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Comparative Mapping of Growth Habit, Plant Height, and Flowering QTLs in Two Interspecific Families of *Leymus*

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ABSTRACT

***Leymus cinereus* (Scribn. & Merr.) Á. Löve and *L. triticoides* (Buckley) Pilg. are tall caespitose and short rhizomatous perennial Triticeae grasses, respectively. Circumference of rhizome spreading, proportion of bolting culms, anthesis date, and plant height were evaluated in two mapping families derived from two interspecific hybrids of *L. cinereus* Acc:636 and *L. triticoides* Acc:641 accessions, backcrossed to one *L. triticoides* tester. Two circumference, two bolting, and two height QTLs were homologous between families. Two circumference, seven bolting, all five anthesis date, and five height QTLs were family specific. Thus, substantial QTL variation was apparent within and between natural source populations of these species. Two of the four circumference QTLs were detected in homoeologous regions of linkage groups 3a and 3b in both families, indicating that one gene may control much of the dramatic difference in growth habit between these species. A major height QTL detected in both families may correspond with dwarfing mutations on barley 2H and wheat 2A. The *L. cinereus* parent contributed negative alleles for all four circumference QTLs, five of nine bolting QTLs, two of five anthesis date QTLs, and one of seven height QTLs. Coupling of synergistic QTL allele effects within parental species was consistent with the divergent growth habit and plant height of *L. cinereus* and *L. triticoides*. Conversely, antagonistic QTL alleles evidently caused transgressive segregation in reproductive bolting and flowering time.**

Leymus wildryes are long-lived Triticeae grasses, closely related to wheat (*Triticum* spp.) and barley (*Hordeum vulgare* L.). The genus *Leymus* includes about 30 species distributed throughout temperate regions of Europe, Asia, and the Americas (Dewey, 1984). More than half of all *Leymus* species are allotetraploids ($2n = 4x = 28$), but octoploid ($2n = 8x = 56$) and dodecaploid ($2n = 12x = 84$) variants may arise from interspecific hybrids (Anamthawat-Jónsson and Bödvarsdóttir, 2001) or autoduplication within species. These cool-season grasses display remarkable variation in growth habit and stature with unusual adaptation to harsh polar, desert, saline, and erosion-prone environments. *Leymus triticoides* (creeping or beardless wildrye) and *L. cinereus* (basin wildrye) are closely related but morphologically divergent North American range grasses. Aggressive rhizomes and adaptation to poorly drained alkaline sites, primarily within the western USA, characterize sod-forming *L. triticoides* (0.3–0.7 m). Conversely, *L. cinereus* is a tall (up to 2 m) conspicuous bunchgrass adapted to deep well-drained soils from Saskatchewan to British Columbia, south to California, northern Arizona, and New Mexico, and east to South Dakota and Minnesota. Most populations of *L. cinereus* and *L. triticoides* are allotetraploids; however, octoploid forms of *L. cinereus* are typical in the Pacific Northwest. Basin wildrye, and octoploid giant wildrye [*L. condensatus* (J. Presl) Á. Löve], are the largest cool-season bunch grasses native to western North America. Artificial hybrids of *L. cinereus*, *L. triticoides*, and other North American *Leymus* wildryes display regular meiosis and stainable pollen (Stebbins and Walters, 1949; Dewey, 1972; Hole et al., 1999). Both *L. cinereus* and *L. triticoides* are highly self-sterile (Jensen et al., 1990) and hybridize with each other in nature. These species are naturally important forage and hay grasses in the Great Basin and other regions of western North America.

Growth habit is a highly variable and ecologically important trait in perennial grasses. Aggressive rhizomes characterize quackgrass [*Elymus repens* (L.) Desv. ex Nevski] and johnsongrass [*Sorghum bicolor* (L.) Moench], which rank among the world's worst perennial grass weeds (Holm et al., 1977). In general, caespitose (i.e., growing in bunches or tufts) and rhizomatous grasses dominate semiarid and mesic grasslands, respectively (Sims et al., 1978). Nutrient islands beneath caespitose grasses may also contribute to clone fitness in this growth form in both mesic and semiarid communities, whereas the distribution of rhizomatous grasses may be restricted to microsites characterized by higher soil organic carbon and nitrogen concentrations (Derner and Briske, 2001). We have observed *L. cinereus* and *L. triticoides* growing in close proximity in mixed stands, at several disturbed sites, with no apparent difference in microhabitat. Conversely, we have observed *L. triticoides* in riparian or wet zones and *L. cinereus* inhabiting dry adjacent uplands, restricted to seemingly different natural microhabitats. In any case, *L. cinereus* and *L. triticoides* display profound differences in growth habit. Lateral branches of *L. cinereus* grow strictly upward, often within the lower leaf sheaths as tillers, whereas the lateral branches of *L. triticoides* frequently grow horizontally or downward under the soil surface as rhizomes. In addition to obvious morphological

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Abbreviations: AFLP, amplified fragment length polymorphism; ANTH, anthesis date; BOLT, reproductive bolting; CIRC, plant circumference; HGHT, plant height; IM, interval mapping; MQM, multiple-QTL model; QTL, quantitative trail locus (i); RFLP, restriction fragment length polymorphism; rMQM, restricted MQM mapping; STS, sequence-tagged site; SSR, simple-sequence repeat.

differences in growth habit and stature, *L. cinereus* and *L. triticoides* display differences in salt tolerance, seed dormancy, seed color, seed shattering, mineral content, tillering, texture, and other characteristics.

The F_1 hybrids of *L. cinereus* and *L. triticoides* are robust and vigorous plants with relatively large biomass potential compared to other range grasses of western North America. Wu et al. (2003) recently constructed high-density molecular genetic linkage maps for two full-sib families, TTC1 and TTC2. The 164-sib TTC1 and 170-sib TTC2 families were derived from two different *L. triticoides* × *L. cinereus* F_1 hybrids, TC1 and TC2, crossed to different clones of the same *L. triticoides* tester genotype (T–tester). The TC1 and TC2 F_1 hybrids were derived from naturally heterogeneous *L. triticoides* and *L. cinereus* seed accessions from Oregon and Alberta, respectively. Together, the TTC1 and TTC2 families capture a snippet of the divergence among and heterogeneity within the *L. triticoides* and *L. cinereus* source populations. These two experimental populations provide unique system for gene discovery research and development of breeding markers in perennial grasses. A close relative of quackgrass, *Leymus* also provides a useful system to study weedy traits such as rhizomes. Our primary objective here was to identify, characterize, and compare QTLs controlling growth habit, plant height, and flowering traits. Another major objective was to compare the location of these *Leymus* QTLs with genomic regions controlling related traits in other cereal and grass species.

MATERIALS AND METHODS

Plant Materials and Genetic Maps

The full-sib TTC1 and TTC2 molecular genetic maps and pedigrees were described by Wu et al. (2003). The 164-sib TTC1 map included 1069 AFLP markers and 38 anchor loci in 14 linkage groups spanning 2001 cM. The 170-sib TTC2 map contained 1002 AFLP markers and 36 anchor loci in 14 linkage groups spanning 2066 cM. Some 488 homologous AFLP loci and 24 anchor markers, detected in both families, showed similar map order among 14 homologous linkage groups of the TTC1 and TTC2 families (Fig. 1). The 14 homologous linkage groups of the allotetraploid TTC1 and TTC2 families were tentatively numbered according to the seven homoeolgous groups of the Triticeae grasses on the basis of synteny of two or more anchor markers from each of the seven homoeologous groups of wheat and barley. Moreover, genome-specific STS markers were used to distinguish the Ns and Xm marker sequences for homoeologous groups four and five on the basis of similarity to *Psathyrostachys juncea* (Fisch.) Nevski (genome designation Ns), which is one of the diploid ancestors of allotetraploid *Leymus* (Wu et al., 2003). Otherwise, homoeologous chromosomes of allotetraploid *Leymus* were arbitrarily distinguished by the letters "a" or "b." For example, LG3a is allegedly homoeologous with LG3b (Fig. 1), an assertion supported by synteny among 10 of the 11 anchor markers mapped to these linkage groups (Wu et al., 2003). Additional SSR and STS anchor markers described in the results below were analyzed by methods described by Wu et al. (2003). Detailed genetic maps containing all 1583 AFLP markers mapped in the TTC1 and/or TTC2 families are shown in Supplementary Data files online. For essential comparisons of homology between the *Leymus* TTC1 and TTC2 families and comparisons of homoeology with other species, we showed all anchor makers but only those AFLP markers that were detected (homologous) in both TTC1 and TTC2 families (Fig. 1).

Field Evaluations

Ramets from each of the two mapping families (TTC1 and TTC2) were space planted in a randomized complete block (RCB) design with two replicates (blocks) per family at the Utah Agriculture Experiment Station Richmond Farm (Cache Co., UT). Each block contained one entry of each of the 164 TTC1 siblings or one clone from each of the 170 TTC2 siblings plus the parental clones (i.e., TC1, TC2 and T) and single-plant representatives of the heterogeneous *L. cinereus* Acc:636 and *L. triticoides* Acc:641 accessions. Individual ramets were transplanted from soil containers (4-cm diameter) in the spring of 2001 to field plots with 2-meter row spacing and 2-meter spacing within rows (2-m centers). Plants were aligned among rows such that each plant had four equidistant (2 m) neighboring plants. Each row contained 34 centers (one plant per center), thus blocks were restricted to six or seven rows. The TTC1 rep1, TTC2 rep1, TTC1 rep2, and TTC2 rep2 blocks were arranged lengthwise, respectively, along the 66-m rows to form one continuous array comprised of 24 × 34 centers (46 × 66 m). Quantitative traits, described below, were measured in 2002, 2003, and 2004.

Rhizome proliferation was measured as plant circumference (CIRC) in late spring or early summer. For caespitose plants, this could be simply measured by stretching a tape ruler around the tussock at the soil surface. The circumference of irregular sods, characteristic of the more rhizomatous plants, was approximated as the perimeter of a polygon where the corners represent the outermost rhizome branches (i.e., the shortest distance around the outermost rhizomes) at the soil surface. Anthesis date (ANTH) was measured as the number of days from 1 January until the first day of anther extrusion, which is most apparent on warm dry mornings, beginning mid-June. In practice, the first day of anther extrusion was interpolated between two or three observations per week. A priori, one unexpected phenomenon in the TTC1 and TTC2 families was variation in reproductive bolting (BOLT), where some genotypes flowered and some did not, first observed in 2002. Individual clones were retrospectively rated as 0 (not flowered) or one (flowered), after seed harvest and mowing, based on ANTH notes take in 2002. We subsequently rated individual plants for the proportion of bolting culms from 0 (no spikes produced) to 1 (virtually all major culms bolting), before seed harvests and mowing, in 2003 and 2004. Plant height (HGHT)as measured after anthesis using a 2-m ruler. Plant height (i.e., actual culm length from soil surface to the spike terminal) was not measured on plants that did not flower.

Data Analysis

QTL analyses were based on trait means from two replicants for each of the 164 TTC1 and 170 TTC2 segregates in 2002, 2003, and 2004. QTL analyses were also performed on averages over all 3 yr, using output from the LSMEANS procedure of SAS (Statistical Analysis Systems Institute Inc., Cary, NC). Coefficients of skewness and kurtosis, g1, and g2 were calculated as described by Snedecor and Cochran (1980). Departures from normality were deemed significant if they exceeded two standard errors of skewness (SES) and kurtosis (SEK) estimated by $(6/N)^{-2}$ and $(24/N)^{-2}$, respectively (Tabachnick and Fidell, 1996). Histograms of phenotypic values

(clonal averages) from 2002, 2003, and 2004 were also prepared for CIRC, BOLT, ANTH, and HGHT (Supplementary Data).

Broad-sense heritabilities within years were obtained using a SAS program for estimating heritability from lines evaluated in an RCB design in a single environment (Holland et al., 2003). Likewise, heritabilities among years were obtained using another SAS program for estimating heritability from lines evaluated in RCB designs in multiple environments (Holland et al., 2003), modified to account for repeated measurements on perennial plants. All class variables (i.e., rep, clone, year) were treated as random effects. Genotypic and phenotypic correlations between traits evaluated using a SAS program for estimating correlations from RCB designs in multiple environments (Holland et al., 2003), also modified to account for repeated measurements. The original SAS programs for estimating heritabilities, genotypic correlations, and phenotypic correlations (Holland et al., 2003) are available at http://www4.ncsu.edu/~jholland/homepage_files/Page571.htm (verified 24 July 2006).

A sequential and reiterative procedure of QTL detection was performed using the MAPQTL five package (Van Ooijen, 2004). Genome-wide interval mapping (IM) (Lander and Botstein, 1989; Van Ooijen, 1992) was performed in 1-cM increments to identify putative QTLs and possible cofactors used in a multiple-QTL model (MQM) (Jansen, 1993; Jansen, 1994; Jansen and Stam, 1994). Markers with the highest log-likelihood ratios (i.e., LOD test statistics) for each QTL (no more than one per chromosome) were selected as the initial set of possible MQM cofactors. A backward elimination procedure was applied to this initial set of cofactors using a conservative significance level of 0.001 to ensure the independence of each cofactor. A reiterative process of restricted MQM (rMQM) mapping, which excludes any syntenous cofactors (i.e., cofactors located on the same linkage group that is being scanned), was used to refine the location of rMQM cofactors (QTLs) and identify new rMQM cofactors (QTLs). Moreover conventional MQM mapping was used to detect (or exclude) syntenous QTLs, where that possibility was apparent. Finally, all putative QTLs were also evaluated using the non-parametric Kruskal–Wallis rank sum test, equivalent to the two-sided Wilcoxon rank sum test for two genotype classes (this study), which means that no assumptions are being made for the probability distributions of the quantitative traits (Lehmann, 1975).

A threshold of 3.3 LOD was used throughout these QTL, MQM, and rMQM detection procedures as a close approximation for a genome-wide 5% significance level as determined from simulation tables based on genome size and family type

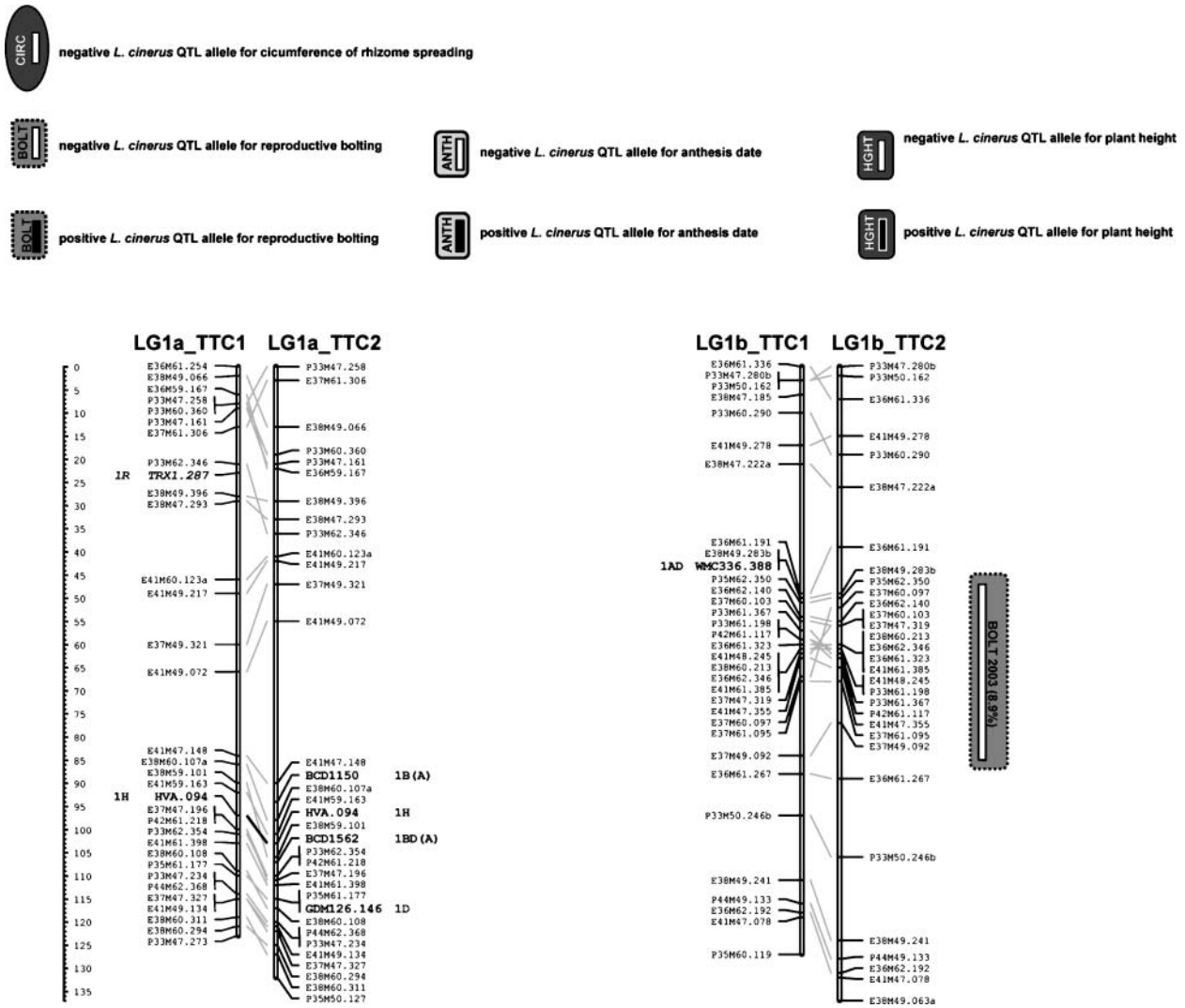
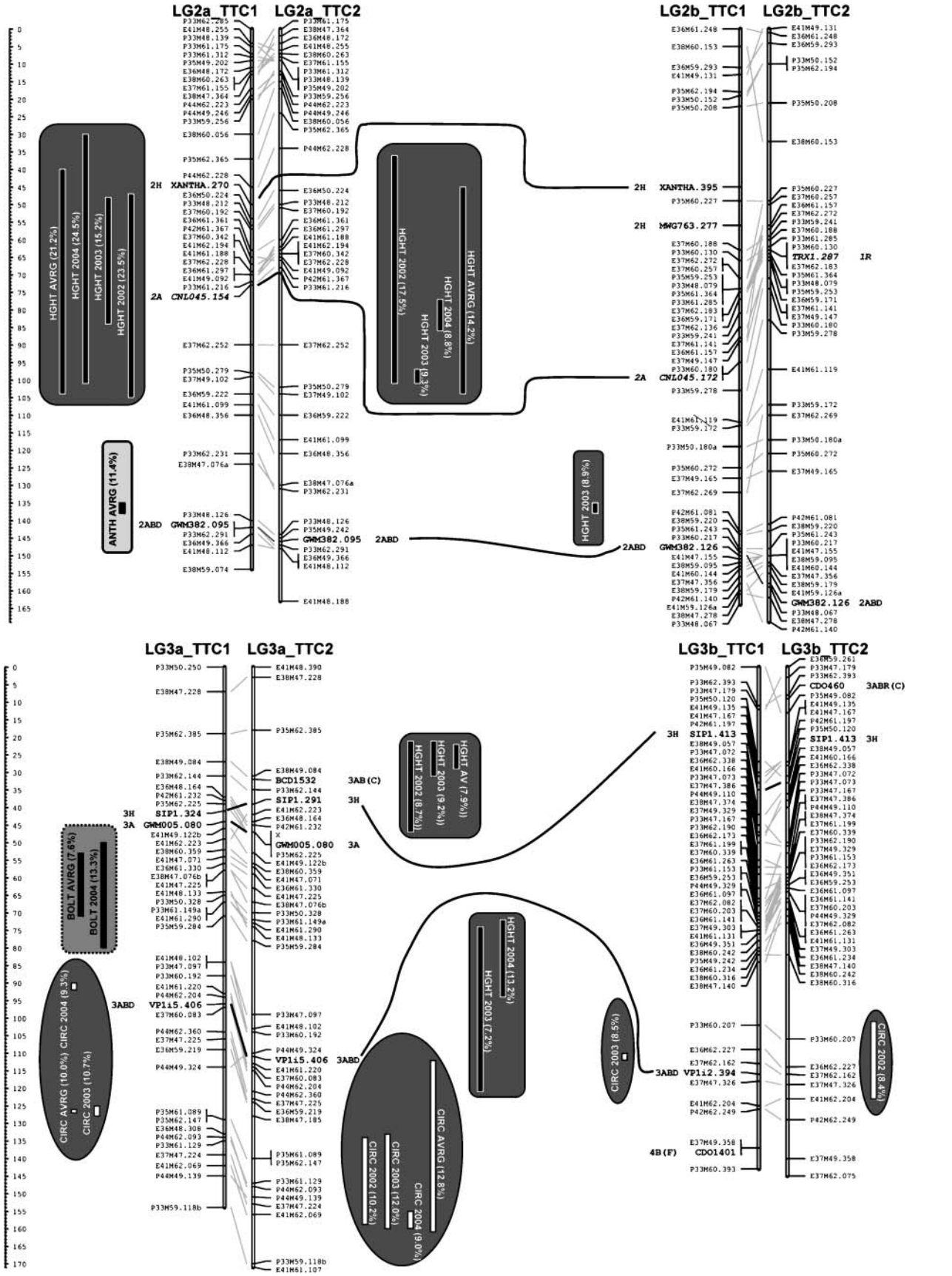


Fig. 1. Continued on next page.



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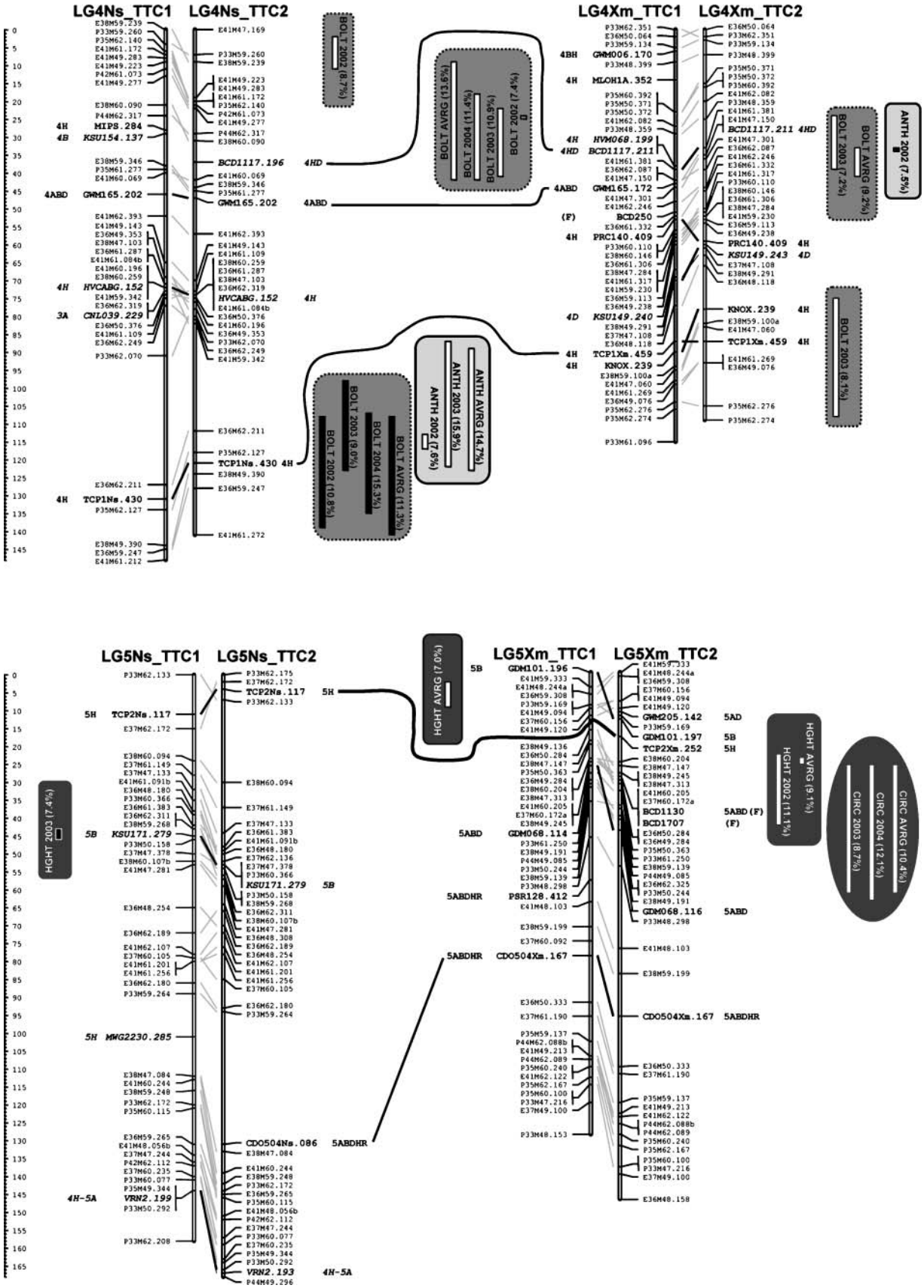

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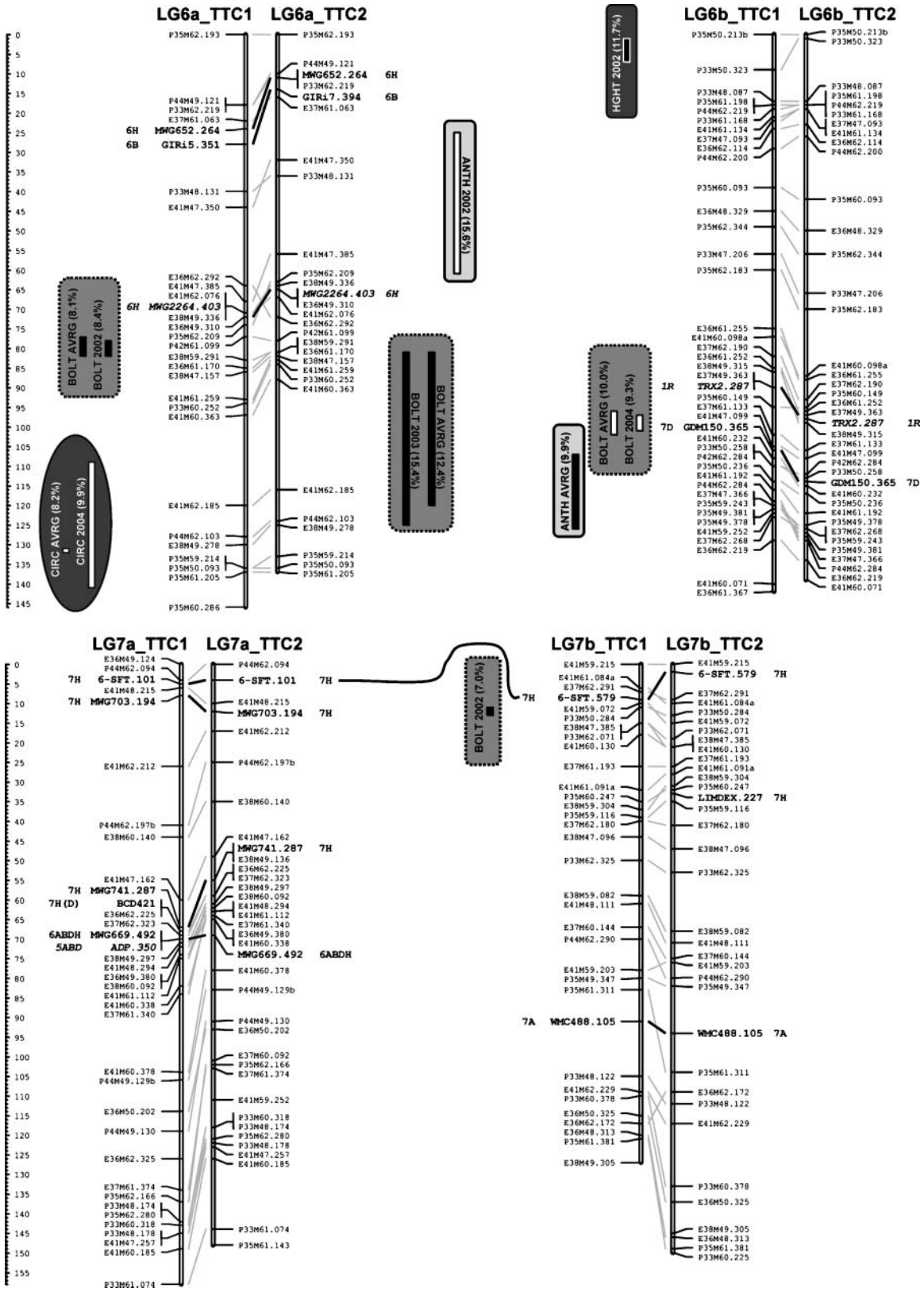

Fig. 1. Summary and comparison of plant height, growth habit, and flowering QTLs detected in the full-sib *Leymus triticoides* × (*L. triticoides* × *L. cinereus*) TTC1 and TTC2 families based on 2002, 2003, 2004, and average measurements. The approximate location of QTLeffects (LOD 3.3), corresponding to a genome-wide $P \leq 0.05$ signficance level, detected using a restricted multiple QTL model (rMQM) are indicated by black or white bars for each trait × year. The updated molecular genetic linkage maps include 488 homologous AFLP markers and mapped in both TTC1 and TTC2 families (Wu et al., 2003), 50 anchor markers (larger bold marker text) mapped in TTC1 and/or TTC2 families (Wu et al., 2003), and 17 additional anchor markers (larger bold italic marker text) mapped in the TTC1 and/or TTC2 families. Annotation next to each anchor marker indicate homoeologous groups of barley (H), wheat (ABD), cereal rye (R), and in parentheses oat chromosome designations.

(Van Ooijen, 1999) and empirical thresholds based on permutation analyses with 1000 replications (Churchill and Doerge, 1994) specific to each trait. Both methods (Van Ooijen, 1999; Churchill and Doerge, 1994) produced remarkably similar results for all four traits evaluated in this study.

Genome-wide IM and rMQM LOD scans based on 2002, 2003, 2004, and average phenotypic values were performed for CIRC, BOLT, ANTH, and HGHT using MapChart version 2.1 (Voorrips, 2002) and the updated *Leymus* TTC1 and TTC2 genetic maps as described in the results section below. Detailed graphs of each genome-wide LOD scan using the complete high-density linkage maps are provided in the Supplementary Data Online.

RESULTS

Molecular Map Update

A subset of 48 CSU or KSU wheat SSR primer pairs described by Yu et al. (2004), the HVCABG SSR primers described by Becker and Heun (1995), and the HVM068 SSR primers described by Liu et al. (1996) were tested for amplification and polymorphism among the parental genotypes. Seven of these SSR primer pairs (CNL45, KSU154, CNL39, HVCABG, HVN068, KSU149, and KSU171) detected 11 loci in the *Leymus* TTC1 and/or TTC2 families (Fig. 1). Likewise, another subset of 48 published STS primer pair sequences (Mano et al., 1999; Taylor et al., 2001; Lem and Lallemand, 2003), three sets of STS primers designed from the wheat VRN2 (Yan et al., 2004), and one set of STS primers designed from the BCD1117 barley cDNA clone (Heun et al., 1991) were also tested for amplification and polymorphism among the parental genotypes. Seven of these STS primer pairs (TRX1, TRX2, BCD1117, MWG2230, VRN2, MWG2264, and ADP) detected 13 loci in the *Leymus* TTC1 and/or TTC2 families (Fig. 1).

The thioredoxin h RFLP marker, *Xbm2*, maps near the self-incompatibility gene locus, S, on homeologous group 1R of *Secale* (Korzun et al., 2001), and thioredoxin h STS markers have been utilized in perennial ryegrass (Taylor et al., 2001) and Kentucky bluegrass (Patterson et al., 2005). The thioredoxin h STS primers used by Patterson et al. (2005) amplified two distinct sets of sequences, designated TRX1 (AY943821 and AY943822 from *L. cinereus* Acc:636; AY943823 and AY943824 from *L. triticoides* Acc:641) and TRX2 (AY943825-AY943827 from *L. cinereus* Acc:636; AY943828-AY943830 from *L. triticoides* Acc:641). We designed TRX1 and TRX2 primers that would amplify corresponding sequences from *L. cinereus* but not *L. triticoides*—thus providing informative polymorphisms for mapping in the TTC1 and TTC2 backcross families. The TRX2 amplicons mapped to homologous positions of the LG6b linkage group, evident by the colinearity with homologous TTC1 and TTC2 AFLP markers (Fig. 1). The TRX1 primers amplified 289 bp sequences that mapped to LG1a in TTC1 and LG2b in TTC2 (Fig. 1). The TRX1 locus on LG1a is presumably homeologous to the *Xbm2* locus on *Secale* 1R. The role of thioredoxin h genes in seed germination, self-incompatibility, grain quality, and characteristics has been evaluated in cereals, grasses, and other plants (Juttner et al., 2000; Langridge et al., 1999; Li et al., 1994, 1997; Sahrawy et al., 1996).

Of special interest with regard to flowering traits, the *VRN1* and *VRN2* genes are the two most potent genes controlling differences in vernalization requirement between winter annual and spring annual barley, wheat, and rye (Dubcovsky et al., 1998), and both of these genes have been cloned (Yan et al., 2003, 2004). The *VRN2* gene is present on homoeologous regions of 4H in winter barleys (evidently absent in most spring barleys) and a 4A/5A translocation region of *Triticum monococcum* 5A (Dubcovsky et al., 1998; Yan et al., 2004). Primers designed from the wheat *VRN2* gene amplified mixed sequences from *Leymus* that were very similar in size (supplemental data) and sequence (DQ486013) to the wheat *VRN2* gene. These putative *Leymus* VRN2 sequences map to a locus on the distal long arm of *Leymus* 5Ns (Fig. 1), which evidently corresponds to the *VRN2* locus on *Triticum monococcum* 5A. Although we have not yet mapped the *Leymus* VRN1 sequences, this gene is closely associated with the CDO504 marker in other Triticeae species and also maps to *Leymus* LG5Ns and LG5Xm (Fig. 1) (Wu et al., 2003).

In summary, seven SSR primer pairs and seven STS primer pairs (supplemental data) detected 15 additional anchor loci in the TTC1 family and nine additional anchor loci in the TTC2 family, not previously described by Wu et al. (2003). The updated TTC1 map includes 1069 AFLP markers and 53 anchor loci in 14 linkage groups spanning 2001 cM. The updated TTC2 map contained 1002 AFLP markers and 45 anchor loci in 14 linkage groups spanning 2066 cM. Some 488 homologous AFLP loci and 31 anchor markers have been mapped in both families, showing similar map order. Thus, 1583 AFLP markers and 67 different anchor loci have been mapped into 14 linkage groups, which evidently correspond to the 14 chromosome pairs of allotetraploid *Leymus* ($2n = 4x = 28$). The map locations of the 17 new anchor loci (24 total including seven homologous pairs) (Fig. 1) were largely consistent with the other anchor loci previously mapped in *Leymus* (Wu et al., 2003).

Growth Habit

The *L. cinereus* Acc:636 and *L. triticoides* Acc:641 progenitor accessions displayed very different levels of rhizomatous spreading, which became more pronounced each year (Table 1). Although CIRC measurements of the TC1 and TC2 F_1 hybrids were significantly greater than *L. cinereus* Acc:636, these means were well-below the midparent, TTC1, and TTC2 averages especially in 2004 (Table 1).

Circumference of plant (rhizome) spreading showed relatively strong broad-sense heritabilities in the TTC1 family and moderate heritabilities in the more rhizomatous TTC2 family (Table 2). The CIRC trait also showed especially strong heritabilities over years, in both TTC1 and TTC2 families; however, this is expected because plant spreading is a cumulative trait. Not surprisingly, the ratio of genotype × year to phenotypic variance was negligible (0.02) for the CIRC trait. The CIRC trait was weakly correlated with the BOLT and ANTH flowering

Table 1. Trait means ± SD for circumference of plant spreading (CIRC), proportion of bolting culms (BOLT), anthesis date (ANTH), and plantheight (HGHT) for *Leymus cinereus* Acc:636, *L. triticoides* Acc:641, *L. triticoides* T parental genotype, interspecific TC1 and TC2 parental hybrids, and full-sib *L. triticoides* × (*L. triticoides* × *L. cinereus*) TTC1 and TTC2 mapping families.

Trait	Year	Acc:636 (n = 18)†	Acc:641 (n = 15)†	t tester (n = 13)‡	TC1 (n = 14)‡	TC2 (n = 14)‡	TTC1 (n = 164)§	TTC2 (n = 168)§
CIRC	2002	23 ± 10	354 ± 186	487 ± 61	96 ± 35	125 ± 37	180 ± 91	299 ± 116
	2003	51 ± 26	594 ± 326	853 ± 120	200 ± 32	231 ± 38	288 ± 122	449 ± 154
	2004	76 ± 21	816 ± 416	1263 ± 177	256 ± 39	282 ± 53	362 ± 137	548 ± 170
	Avg.	57 ± 23	702 ± 208	653 ± 112	183 ± 28	212 ± 40	273 ± 112	430 ± 144
BOLT	2002	0.28 ± 0.46	0.67 ± 0.48	1.00 ± 0.00	0.79 ± 0.43	1.00 ± 0.00	0.57 ± 0.43	0.73 ± 0.38
	2003	0.63 ± 0.43	0.56 ± 0.32	0.98 ± 0.06	0.88 ± 0.19	0.77 ± 0.21	0.33 ± 0.24	0.36 ± 0.25
	2004	0.85 ± 0.33	0.78 ± 0.35	1.00 ± 0.00	0.84 ± 0.19	0.81 ± 0.23	0.55 ± 0.26	0.67 ± 0.27
	Avg.	0.60 ± 0.58	0.74 ± 0.24	0.99 ± 0.03	0.83 ± 0.14	0.85 ± 0.09	0.49 ± 0.26	0.59 ± 0.26
ANTH	2002	176.8 ± 2.1	173.3 ± 2.6	172.0 ± 0.0	173.6 ± 2.1	173.1 ± 1.5	173.5 ± 1.9	173.8 ± 4.8
	2003	171.1 ± 5.1	169.3 ± 1.4	169.0 ± 0.0	169.0 ± 0.0	169.0 ± 0.0	172.1 ± 5.7	171.3 ± 4.9
	2004	178.3 ± 3.9	172.6 ± 1.6	173.0 ± 0.0	175.9 ± 4.0	175.9 ± 4.0	175.7 ± 4.6	174.1 ± 3.7
	Avg.	175.9 ± 2.4	172.2 ± 1.1	171.3 ± 0.0	172.8 ± 1.3	172.6 ± 1.5	174.5 ± 3.5	173.4 ± 4.0
HGHT	2002	99.0 ± 39.8	94.5 ± 12.6	100.8 ± 5.7	145.8 ± 15.9	141.8 ± 10.6	103.9 ± 13.8	109.7 ± 13.0
	2003	135.6 ± 23.0	98.5 ± 8.5	100.9 ± 5.9	155.1 ± 12.6	138.4 ± 13.3	108.3 ± 12.9	106.1 ± 12.4
	2004	182.9 ± 18.5	123.3 ± 12.7	125.7 ± 3.4	184.7 ± 12.3	164.6 ± 8.7	134.8 ± 13.8	137.8 ± 11.8
	Avg.	142.2 ± 26.0	107.3 ± 8.7	109.2 ± 2.4	161.8 ± 11.2	148.3 ± 8.3	116.1 ± 12.5	117.9 ± 10.7

Measurements expressed in cm (CIRC and HGHT), proportion (BOLT), and days from January one (ANTH).
† Sample size based on individual plants.
‡ Sample size based on individual clones of each parental genotype.
§ Sample size based on means of two clones for each genotype (n).

traits, in the TTC1 and/or TTC2 families, but virtually independent from plant height in both TTC1 and TTC2 families (Table 3).

At least three significant CIRC QTLs were detected in each family; however, not more than two QTLs were significant in any one year and population (Table 4, Fig. 1). The strongest, most consistent CIRC QTLs in both TTC1 and TTC2 families were located in homologous regions of LG3a (Table 4, Fig. 1). Likewise, homologous CIRC QTLs were also detected on LG3b in both TTC1 and TTC2 families (Fig. 1). The LG3a and LG3b CIRC QTLs, detected in both TTC1 and TTC2 families, are all located near homoeologous copies of VP1 gene markers (Fig. 1). The transcription factor *Viviparous-1* (encoded by the *Vp1* gene) induces and maintains seed dormancy (McCarty et al., 1991) and has been mapped to orthologous loci in wheat, maize, and rice (Bailey et al., 1999). In addition to the homologous and possibly homoeologous QTLs detected on LG3a and LG3b, in both TTC1 and TTC2 families, a TTC1-specific CIRC QTL was detected on LG6a and a TTC2-specific CIRC QTL was detected on LG5Xm (Fig. 1). Thus, two CIRC QTLs were common to both TTC1 and TTC2 families and two were unique to only one family. The *L. cinereus* alleles had negative effects at all four (LG3a, LG3b, LG5Xm, and LG6a) CIRC QTLs (Table 4, Fig. 1) consistent with divergent phenotypes of the parental species.

Although significant skewness was detected in several evaluations, all four CIRC QTLs (Table 4, Fig. 1) were highly significant ($P < 0.0001$) on the basis of the nonparametric Kruskal–Wallis rank sum test.

Flowering Traits

The BOLT and ANTH traits showed relatively strong negative genotypic correlations in both TTC1 and TTC2 families (Table 3), evidently controlled by several common (probably pleiotropic) QTLs (Fig. 1). Thus, data from these two traits are presented together in one section.

The *L. cinereus* Acc:636 and *L. triticoides* Acc:641 progenitor accessions; F_1 interspecific hybrids (TC1 and TC2); and TTC1 and TTC2 families displayed similar BOLT and ANTH phenotypic means (Table 1), except for the fact that *L. cinereus* showed relatively weak bolting in the first establishment year. *Leymus cinereus* has a relatively large stature, requiring several years to reach reproductive maturity. Yet, the TTC1 and TTC2 families displayed transgressive BOLT and ANTH segregation that persisted through 2002, 2003, and 2004 (see trait distributions in Supplemental Data). Unlike the F_1 hybrids or parental source populations, some transgressive progeny failed to flower in 2002, 2003, 2004, and 2005.

The BOLT and ANTH traits both showed relatively weak plot-mean heritabilities over years (Table 2). The ratio of genotype × year interaction to phenotypic variance was substantially greater for ANTH (0.18) and BOLT (0.21) compared with CIRC (0.02) or HGHT (0.13), which explains the low plot-mean heritabilities

Table 2. Estimates of broad-sense heritability (H^2) ± SE based on a plot basis and, in parentheses, genotypic mean basis for circumference of plant spreading (CIRC), proportion of bolting culms (BOLT), anthesis date (ANTH), and plant height (HGHT) in the full-sib *Leymus triticoides* × (*L. triticoides* × *L. cinereus*) TTC1 and TTC2 mapping families.

Trait	Family	2002	2003	2004	Over years
CIRC	TTC1	0.72 ± 0.04 (0.84 ± 0.03)	0.77 ± 0.03 (0.87 ± 0.02)	0.73 ± 0.04 (0.85 ± 0.02)	0.64 ± 0.04 (0.91 ± 0.01)
	TTC2	0.54 ± 0.06 (0.70 ± 0.05)	0.55 ± 0.06 (0.71 ± 0.05)	0.57 ± 0.05 (0.73 ± 0.04)	0.50 ± 0.05 (0.86 ± 0.03)
BOLT	TTC1	0.44 ± 0.06 (0.61 ± 0.06)	0.63 ± 0.05 (0.77 ± 0.04)	0.64 ± 0.05 (0.77 ± 0.04)	0.38 ± 0.04 (0.76 ± 0.04)
	TTC2	0.43 ± 0.06 (0.61 ± 0.06)	0.64 ± 0.05 (0.78 ± 0.04)	0.69 ± 0.04 (0.81 ± 0.03)	0.41 ± 0.04 (0.78 ± 0.03)
ANTH	TTC1	0.50 ± 0.07 (0.67 ± 0.07)	0.42 ± 0.08 (0.59 ± 0.08)	0.51 ± 0.06 (0.67 ± 0.06)	0.31 ± 0.05 (0.71 ± 0.05)
	TTC2	0.73 ± 0.04 (0.84 ± 0.03)	0.43 ± 0.08 (0.60 ± 0.07)	0.46 ± 0.07 (0.63 ± 0.06)	0.41 ± 0.04 (0.78 ± 0.03)
HGHT	TTC1	0.69 ± 0.05 (0.81 ± 0.03)	0.51 ± 0.07 (0.68 ± 0.06)	0.73 ± 0.04 (0.84 ± 0.03)	0.54 ± 0.04 (0.86 ± 0.02)
	TTC2	0.66 ± 0.05 (0.79 ± 0.03)	0.53 ± 0.06 (0.69 ± 0.06)	0.59 ± 0.05 (0.75 ± 0.04)	0.48 ± 0.04 (0.82 ± 0.03)

Table 3. Genotypic and in parentheses phenotypic trait correlations ± SE for circumference of plant spreading (CIRC), proportion of bolting culms (BOLT), anthesis date (ANTH), and plant height (HGHT) in the full-sib *Leymus triticoides* (*L. triticoides* × *L. cinereus*) TTC1 (below diagonal) and TTC2 (above diagonal) families.

	CIRC	BOLT	ANTH	HGHT
CIRC	—	0.24 ± 0.09 (0.13 ± 0.05)	−0.22 ± 0.09 (−0.10 ± 0.05)	0.12 ± 0.09 (0.02 ± 0.05)
BOLT	0.22 ± 0.08 (0.15 ± 0.05)	—	−0.83 ± 0.04 (−0.56 ± 0.03)	0.32 ± 0.09 (0.36 ± 0.04)
ANTH	−0.33 ± 0.09 (−0.22 ± 0.05)	−0.81 ± 0.06 (−0.48 ± 0.03)	—	−0.26 ± 0.09 (−0.32 ± 0.04)
HGHT	0.01 ± 0.09 (0.04 ± 0.06)	0.32 ± 0.09 (0.40 ± 0.04)	0.07 ± 0.11 (−0.19 ± 0.05)	—

over years for the ANTH and BOLT flowering traits. Although, BOLT and ANTH traits show significant genotype × year interaction and the significance of QTLs vary by year (Tables 4), several QTLs were detectable over years (Fig. 1). One notable exception was a highly significant TTC2 ANTH QTL on LG6a in 2002, which was virtually nonexistent in subsequent years. Bolting deficiencies were strongly correlated with late flowering and weakly correlated with short caespitose characteristics (Table 3).

A total of nine BOLT QTLs were detected in the TTC1 and/or TTC2 families (Table 4, Fig. 1). The TTC1 and TTC2 BOLT QTL peaks on the upper portion of LG4Xm overlap well enough (Fig. 1) that we counted these as one homologous QTL. Likewise, we believe that there is insufficient separation of the TTC1 and TTC2 BOLT QTLs on LG6a to declare these as different (Fig. 1). Thus, *L. cinereus* contributed five negative TTC1 and/or TTC2 BOLT QTL alleles and four positive TTC1 and/or TTC2 BOLT QTL alleles (Table 4, Fig. 1). Two of these nine BOLT QTLs were significant in both TTC1 and TTC2 families (i.e., homologous), whereas seven QTLs were unique to TTC1 or TTC2. Four QTLs explained up to 43.9% of the BOLT variation in the 2003 TTC2 data set, which was the most explanatory QTL model in this study (Table 4).

A total of five significant ANTH QTLs were detected in the TTC1 or TTC2 families (Table 4, Fig. 1). All five significant ANTH QTLs were unique to TTC1 or TTC2 (Table 4 and Fig. 1). However, the TTC1 family displayed

Table 4. Summary of QTL effects detected using multiple QTL model (MQM) scans of circumference of plant spreading (CIRC), proportion of bolting culms (BOLT), anthesis date (ANTH), and plant height (HGHT) in the full-sib *Leymus triticoides* × (*L. cinereus* × *L. triticoides*) TTC1 and TTC2 backcross mapping families.

Trait	Family	Position (interval†)	2002 LOD (R^2, effect‡)	2003 LOD (R^2, effect‡)	2004 LOD (R^2, effect‡)	Average LOD (R^2, effect‡)
CIRC	**TTC1**	**3a (125–137)**	**NS**	**3.88 (10.7%, −79)§**	**3.16 (9.3%, −83)§**	**3.41 (10.0%, −69)¶**
		3b (111–112)	**NS**	**3.47 (8.5%, −73)¶**	**NS**	**NS**
		6a (107–144)	**NS**	**NS**	**4.08 (9.9%, −87)§**	**3.33 (8.2%, 64.4)§**
		Overall R^2	**NS**	**16.9%**	**18.4%**	**16.4%**
	TTC2	**3a (111–161)**	**4.36 (10.2%, −75)§**	**5.15 (12.0%, −107)§**	**3.9 (9.0%, −102)§**	**5.44 (12.8%, −104)§**
		3b (111–144)	**3.56 (8.4%, −68)§**	**NS**	**NS**	**NS**
		5Xm (25–66)	**NS**	**3.84 (8.7%, −94)§**	**4.76 (12.1%, −121)§**	**4.49 (10.4%, −96)§**
		Overall R^2	**19.7%**	**21.0%**	**20.3%**	**20.4%**
BOLT	**TTC1**	**3a (27–80)**	**NS**	**NS**	**6.12 (13.3%, +0.19)§**	**4.00 (7.6%, +0.14)¶**
		4Xm (11–42)	**3.17 (7.4%, −0.24)§**	**4.12 (10.9%, −0.16)§**	**5.12 (11.4%, −0.18)§**	**6.33 (13.6%, −0.19)§**
		6a (61–88)	**3.57 (8.4%, +0.25)§**	**NS**	**NS**	**4.01 (8.1%, +0.15)§**
		6b (95–117)	**NS**	**NS**	**3.47 (9.3%, −0.16)¶**	**4.11 (10.0%, −0.16)§**
		7b (11–15)	**3.20 (7.0%, +0.24)§**	**NS**	**NS**	**NS**
		Overall R^2	**25.7%**	**10.9%**	**28.9%**	**35.9%**
	TTC2	**1b (47–85)**	**NS**	**5.38 (8.9%, −0.15)§**	**NS**	**NS**
		4Ns (0–24)	**3.78 (8.7%, −0.25)§**	**NS**	**NS**	**NS**
		4Ns (95–142)	**4.36 (10.8%, +0.26)§**	**4.53 (9.0%, +0.16)§**	**5.65 (15.3%, +0.21)§**	**5.64 (11.3%, +0.18)§**
		4Xm (23–41)	**NS**	**4.05 (7.2%, −0.13)§**	**NS**	**4.37 (9.2%, −0.16)§**
		4Xm (68–109)	**NS**	**4.61 (8.1%, −0.14)¶**	**NS**	**NS**
		6a (80–125)	**NS**	**7.33 (15.4%, +0.20)§**	**NS**	**4.89 (12.4%, +0.18)§**
		Overall R^2	**19.9%**	**43.9%**	**15.3%**	**32.6%**
ANTH	**TTC1**	**2a (121–139)**	**NS**	**NS**	**NS**	**4.59 (11.4%, +2.5)§**
		6b (108–127)	**NS**	**NS**	**NS**	**4.00 (9.9%, +2.2)¶**
		Overall R^2	**NS**	**NS**	**NS**	**19.2%**
	TTC2	**4Ns (87–124)**	**3.54 (7.6%, −2.7)§**	**5.21 (15.9%, −4.5)§**	**NS**	**5.42 (14.7%, −3.12)§**
		4Xm (32–34)	**3.70 (7.5%, +2.7)§**	**NS**	**NS**	**NS**
		6a (108–127)	**5.85 (15.6%, −3.9)§**	**NS**	**NS**	**NS**
		Overall R^2	**30.9%**	**13.7%**	**NS**	**14.7%**
HGHT	**TTC1**	**2a (30–105)**	**7.66 (23.5%, +13.3)§**	**6.74 (15.2%, +10.1)§**	**10.8 (24.5%, +13.6)§**	**10.36 (21.2%, +11.6)§**
		2b (130–164)	**NS**	**NS**	**5.16 (10.7%, +9.0)§**	**5.07 (9.6%, +7.8)§**
		5Ns (42–44)	**NS**	**3.44 (7.4%, +7.0)¶**	**NS**	**NS**
		5Xm (0–11)	**NS**	**NS**	**NS**	**3.77 (7.0%, −6.8)[e]**
		6b (0–24)	**NS**	**3.86 (11.7% +9.1)§**	**NS**	**NS**
		Overall R^2	**20.6%**	**31.4%**	**33.8%**	**38.8%**
	TTC2	**2a (37–103)**	**8.56 (17.5%, +11.1)§**	**4.20 (9.3%, +7.6)§**	**3.50 (8.8%, +7.0)§**	**6.40 (14.2%, +8.1)§**
		3a (20–34)	**4.10 (8.7%, +7.74)¶**	**4.15 (9.2%, +7.5)¶**	**NS**	**3.98 (7.9%, +6.0)§**
		3a (77–89)	**NS**	**3.42 (7.2%, +6.7)§**	**4.96 (13.2%, +8.6)§**	**NS**
		5Xm (6–82)	**5.64 (11.1%, −8.8)§**	**NS**	**NS**	**4.65 (9.1%, −6.9)§**
		Overall R^2	**34.1%**	**28.8%**	**20.3%**	**34.5%**

† Approximate positions (cM) where LOD scan exceeded 3.3 threshold as shown in Fig. 1.

‡ Mean of heterozygous genotype (*L. cinereus* and *L. triticoides* alleles)– mean of homozygous genotypes (two *L. triticoides* alleles) expressed in centimeters (CIRC and HGHT), proportions (BOLT), or days (ANTH).

§ Cofactors selected by interval mapping (LOD > 3.3) and backward elimination (significant at the 0.001 level).

¶ Additional cofactors identified by restricted multiple QTL model mapping (LOD > 3.3) and backward elimination.

ANTH LOD values that were nearly significant on a chromosome region homologous to the TTC2 ANTH QTL on LG4Xm (see ANTH LOD scan in Supplemental Data). Similarly, both TTC1 and TTC2 displayed nearly significant ANTH LOD values on homologous regions of LG3b. In any case, *L. cinereus* contributed three positive and two negative ANTH QTL alleles at the five significant QTLs (Table 4, Fig. 1).

The genome-wide LOD scans were similar but not identical for the BOLT and ANTH flowering traits (Fig. 1 and Supplemental Data). The TTC1 BOLT QTL on LG3a had no apparent effects on ANTH. The TTC2 BOLT QTLs on LG4Ns were associated with significant or nearly significant ANTH QTLs. Likewise, the BOLT QTLs on LG4Xm were associated with significant or nearly significant ANTH QTLs in the TTC1 and TTC2 families. The TTC1 ANTH QTL on LG 5Ns was associated with elevated BOLT LOD, albeit less than the 3.3 LOD threshold (Supplemental Data). Interestingly, the TTC1 and TTC2 BOLT QTLs on LG6a were associated with significant ANTH QTLs only in the TTC2 family. The TTC1 BOLT QTL on LG6a was not associated with elevated ANTH LOD values. The latter observation raises some doubt about the putative homology of the TTC1 and TTC2 BOLT QTLs on LG6a (Fig. 1). Finally the TTC1 BOLT QTLs on LG6b and LG7b were closely associated with a significant or nearly significant ANTH QTLs, respectively.

Significant skewness and kurtosis was detected for BOLT and ANTH; however, all QTLs (Table 4, Fig. 1) were highly significant ($P < 0.0001$) on the basis of the nonparametric Kruskal–Wallis rank sum test.

Plant Height

The *L. cinereus* Acc:636 plants were substantially taller than the *L. triticoides* Acc:641 plants in 2003 and 2004 (Table 1). However, the *L. cinereus* plants require several years to reach full-size; thus, interspecific differences in plant height were not apparent in 2002. Interestingly, the TC1 and TC2 F_1 hybrid genotypes displayed greater plant height than the *L. cinereus* Acc:636 or *L. triticoides* Acc:641 reference individuals, especially in 2002 and 2003 (Table 1). The 2004 HGHT means were substantially greater in 2004, which may be attributable to better rainfall (i.e., 2002 and 2003 were severe drought years) and/or longer plant establishment.

Heritabilities over years (Table 2) for HGHT were intermediate between CIRC and the two flowering traits (ANTH and BOLT). Likewise, the ratio of genotype × year interaction to phenotypic variance for HGHT (0.13) was intermediate between CIRC (0.02) and the flowering traits, ANTH (0.18) and BOLT (0.21). Weak positive genotypic correlations between HGHT and BOLT were detected in TTC1 and TTC2 families, but no other trait correlations with HGHT were detected in both families.

We detected a total of seven HGHT QTLs in the TTC1 and/or TTC2 families (Table 4, Fig. 1). The TTC2 HGHT QTL on LG3a was split into two QTLs using a combination of rMQM and unrestricted MQM mapping (Table 4, Fig. 1). The single largest HGHT QTL was located in homologous regions of LG2a in both TTC1 and TTC2 families. Likewise, overlapping TTC1 and TTC2 HGHT QTLs on LG5Xm were too close to separate (Fig. 1), which we count as one homologous QTL. Moreover, LG5Xm was the only chromosome that contributed negative HGHT QTL alleles from the taller *L. cinereus* species, which also seems to support our interpretation that these are homologous QTLs in the TTC1 and TTC2 families. Thus, *L. cinereus* contributed six positive and one negative HGHT QTL alleles in the TTC1 and/or TTC2 families, which is consistent with divergent phenotypes of the parental species.

DISCUSSION

Leymus Molecular Genetic Maps

Seventeen additional anchor markers described in this study support previous linkage group identifications, tentatively numbered according to the seven homoeologous groups of the wheat, barley, and rye Triticeae cereals (Wu et al., 2003). Among the well-characterized Triticeae genomes, researchers have detected one rearrangement in *Aegilops longissima* Schweinf. & Muschl. (S^I genome), 11 in *Ae. umbellulata* Zhuk. (U genome), 0 in *Ae. speltoides* Tausch (S genome), seven in *Secale cereale* L. cultivated ryes (R genome), and two paracentric inversions in *H. vulgare* cultivated barleys (H genome) relative to *Ae. tauschii* Coss., the donor of the hexaploid wheat D genome (Devos and Gale, 2000). The A, B, and D genomes of allohexaploid wheat are evidently colinear except for several large reciprocal translocations involving chromosome arms 2BS and 6BS and chromosomes 4A, 5A, and 7B (Devos and Gale, 2000). One of these rearrangements involves a reciprocal translocation between 4AL and 5AL, which includes the *VRN2* gene in *T. monococcum* L. (Dubcovsky et al., 1998; Yan et al., 2004). Interestingly, the location of the VRN2 gene on *Leymus* LG5Ns evidently corresponds with the location of *VRN2* in *T. monococcum* (Devos et al., 1995; Dubcovsky et al., 1996). Thus, the *Leymus* Ns and *T. monococcum* genomes evidently share the same 4AL/5AL translocation arrangement. Although we detected seven instances of marker synteny in *Leymus* that were not syntenous in other Triticeae species, we have no firm evidence of rearrangements other than the putative 4Ns/5Ns translocation that has already been documented in wheat. Incongruent RFLP marker locations can often be attributed to paralogous duplications or, in the case of PCR, priming annealing at nonhomologous loci. Although there is substantial evidence of colinearity between the *Leymus* Ns, *Leymus* Xm, wheat A, wheat B, wheat D, and barley H genomes, we cannot exclude the possibility of a few yet undetected rearrangements in *Leymus.*

These are the first QTL analyses conducted using the high-density linkage maps published by Wu et al. (2003). Throughout much of the linkage map, relatively small irregularities in the LOD scans (Supplemental Data) resulted from imperfect genotyping, missing data, and ambiguous marker orders. Nevertheless, these QTL maps

provide a useful starting point for high-resolution QTL mapping experiments using six advanced backcross populations that are currently being genotyped and evaluated in clonally replicated field trials.

Comparison of TTC1 and TTC2 QTLs

Two circumference, two bolting, and two height QTLs were evidently homologous in both TTC1 and TTC2 families. Conversely, two circumference, seven bolting, all five anthesis date, and five height QTLs were unique to TTC1 or TTC2 families. Differences between the TTC1 and TTC2 families can be attributed to differences between the TC1 and TC2 hybrids, which in turn can only be attributed to genetic variation within the *L. cinereus* Acc:636 and *L. triticoides* Acc:641 natural germplasm sources. Thus, functionally important QTL variation was apparent within and between natural source populations of these experimental families and species.

Growth Habit

The coincidence of QTL effects near the VP1 loci on LG3a and LG3b, in both TTC1 and TTC2, suggest that these QTLs may be controlled by homoeologous copies of the one gene. If so, this gene evidently controls much of the dramatic difference in growth habit between *L. cinereus*, *L. triticoides*, and perhaps other grasses. The only other CIRC QTLs, on LG5Xm and LG6a, were unique to the TTC1 and TTC2 families, respectively. A preponderance of mapped barley mutations (four of six loci) that affect vegetative axillary development have been localized to chromosome 3HL (minus arm), including *absent lower laterals* (*als*), *low number of tillers1* (*int1*), and *semi brachytic* (*usu*), which produce fewer tillers, in addition to *granum-a* (*gra-a*), which produces significantly more tillers (Babb and Muehlbauer, 2003; Franckowiak, 1996). A recessive gravitropic *lazy dwarf* gene (*lzd*) was also mapped to the short arm of barley chromosome 3 (Franckowiak, 1996; Takahashi et al., 1975), but this mutation seems to be more proximal to the centromere than the *Leymus* LG3a and LG3b growth habit QTLs. Chromosome 3 is highly conserved within the Triticeae (Devos and Gale, 2000). Colinear from end to end with rice chromosome 1, Triticeae group three is also the most conserved of all chromosome groups when compared with rice (La Rota and Sorrells, 2004). Rice chromosome 1 contains putative transmembrane auxin efflux carrier and DNAJ-like genes (International Rice Genome Sequencing Project, 2005), near the *Vp1* locus, originally identified in *Arabidopsis pin*-forming (Gälweiler et al., 1998) and *arg1* (altered response to gravity) (Sedbrook et al., 1999) mutants, respectively. However, *pin1*- and *arg1*-like sequences are also present in other regions the rice genome. Rhizome (Hu et al., 2003) and tiller angle (Li et al., 1999) QTLs also map to rice chromosome 1, but these rice loci were not located near the rice *Vp1* gene.

The TTC2 CIRC rMQM QTL on LG5Xm mapped near the TCP-like sequence (TCP2) amplified from *L. triticoides* rhizome cDNA using primers designed from the *teosinte branched one* gene (Doebley et al., 1997), BCD1130, BCD1707, and PSR128 loci. The PSR128 marker also maps in the centromere region of maize chromosome 4 near the recessive *lazy1* (*la1*) gene (Lawrence et al., 2004). Lazy maize mutants, allelic to *la1*, actively grow downward (prostrate) under light (Firn et al., 2000). We found BCD1707 and BCD1130 sequences on rice chromosome 11, using a TBLASTX search (Ware et al., 2002) of the rice genome (International Rice Genome Project, 2005), near another *lazy* (*la*) gene (Miura et al., 2003). The centromere and short arm regions of Triticeae group 5 are homoeologous to rice chromosomes 9 and 12, respectively (Van Deynze et al., 1995; La Rota and Sorrells 2004). Rice chromosome 9 contains a major QTL (*Ta*) for tiller angle (Li et al., 1999).

The TTC1 CIRC QTL peak on LG6a is located approximately (minus) 40 cM from the MWG2264 locus. Interestingly, the *uniculm* (*cul2*) mutation and MWG2264 loci are both located (minus) 8.8 and 3 cM relative to the cMWG679 locus on barley 6H (Babb and Muehlbauer, 2003; Graner et al., 1991; Graner, 2004). Compared with other barley mutants, *uniculm2* (*cul2*) is unique in that it inhibits formation of axillary meristems and does not produce tillers (Babb and Muehlbauer, 2003).

We were not able to identify rhizome QTLs in *Leymus* that were homeologous to rhizome QTLs of *Sorghum* and *Oryza*. Paterson et al. (1995) detected about 12 rhizome QTLs including a conspicuous cluster of QTLs affecting rhizome number, rhizome distance, and seedling tillers on *Sorghum* linkage group C, which corresponds to wheat group 4 (Draye et al., 2001). The latter *Sorghum* QTL also corresponds with sucker and stalk number QTL in *Saccharum* (Jordan et al., 2004), in addition to the *Rhz2* gene and 11 other QTLs controlling rhizome proliferation differences between *O. sativa* L. and *O. longistaminata* A. Chev. & Roehr. (Hu et al., 2003). We did not detect any plant circumference QTLs on *Leymus* LG 4Ns or LG4Xm, which include the PRC140 rhizome QTL marker (Draye et al., 2001) derived from a *Sorghum* LG C and sequences orthologous to the maize *teosinte branched one* gene (Doebley et al., 1997). The *Rhz3* gene and rhizome QTL on *Oryza* chromosome 4 correspond to rhizome QTLs on *Sorghum* LG D (Hu et al., 2003) and *Saccharum* (Jordan et al., 2004). This region of *Oryza* chromosome 4 and *Sorghum* LG D evidently corresponds to Triticeae group 2 (Paterson et al., 1995; Van Deynze et al., 1995). We did not detect any significant rhizome QTL effects on Triticeae group 2.

Bolting and Anthesis Date

The genetic control of flowering time is complex, involving three major groups of genes on all seven Triticeae chromosome groups (Snape et al., 2001). Two of these groups interact with the environment, namely those controlling vernalization (*Vrn*) and photoperiod (*Ppd*). The third set of genes controls developmental rate independent of vernalization and photoperiod, so-called "earliness per se" (*Eps*) genes. A relatively large number of *Eps* genes have been mapped to all seven homoeologous groups of wheat and/or barley (Laurie et al., 1995; Snape

et al., 2001), several of which may correspond to BOLT and/or ANTH QTLs detected in *Leymus*.

The *Vrn* genes map to Triticeae groups 1, 4, 5, and 7 (Dubcovsky et al., 1998; Snape et al., 2001). Moreover, the two most potent genes, on groups 4 and 5, have been cloned and characterized (Yan et al., 2003; Yan et al., 2004). Surprisingly, no significant bolting or anthesis date QTLs were associated with the *Leymus* CDO504 markers on LG5Ns and LG5Xm, which are presumably closely linked with genes that are orthologous to VRN1. We have amplified VRN1 sequences from *Leymus* but have not yet found polymorphisms needed for mapping. Likewise, no significant bolting or anthesis date QTLs were associated with the VRN2 gene on LG5Ns. A second yet undetected VRN2 gene may or may not be present on the Xm genome. The most conspicuous wheat and barley *Ppd* genes are located on homoeologous regions of the 2A, 2B, 2D, and 2H short (plus) chromosome arms. Other *Ppd* genes have also been reported on barley 1H (Laurie et al., 1995) and 6H (Strake and Börner, 1998). We did not detect ANTH or BOLT QTLs on short (minus) arm of *Leymus* group two chromosomes.

The TTC1 and TTC2 families displayed substantial genetic variation for anthesis date and bolting, including genotypes that have not flowered in the field, which was not apparent among the parental species accessions. This transgressive segregation can be explained by a mixture of antagonistic (i.e., positive and negative) QTL alleles from each parental species. *Leymus cinereus* contributed five positive and four negative BOLT QTLs and three positive and two negative ANTH QTLs. Thus, correspondence of bolting and anthesis date QTLs (and relatively strong negative genotypic correlations) suggest that outbreeding depression (i.e., transgressive genotypes that to flower) was associated with flowering (i.e., lateness) genes under balancing selection. Although population means and standard deviations may not demonstrate significant bolting depression, the fact that a substantial number of progeny failed to flower year after year indicates that some outbreeding depression is occurring. We have not observed this phenomenon within the *L. cinereus* Acc:636 or *L. triticoides* Acc:641 natural source populations.

Plant Height

The HGHT QTLs on LG2a displayed remarkably strong effects and similar map locations in both TTC1 and TTC2 families, suggesting that these QTLs are homologous. The LG2a HGHT QTL was the only major positive HGHT effect associated with chromatin of the much taller *L. cinereus* species and detected in both TTC1 and TTC2 families. We interpret these results to mean that this is one of the key QTLs controlling relatively large differences in plant height between *L. cinereus* and *L. triticoides*. Börner et al. (1999) mapped gibberellin-sensitive *gal* (*GA-less*) and gibberellin-insensitive *gai* (*GA-ins*) mutations about 55 cM apart on barley 2H. The *Leymus* LG2a HGHT QTL spans a 40 cM interval from the XANTHA locus down below the CNL045 locus (Fig. 1). The XANTHA–CNL045 LG2b interval also includes the cWMG763 locus (Fig. 1), which was mapped to the short (plus) arm of barley 2H about 36 cm distal from the MWG2287 marker in the barley IGRI × FRANKA population (Graner et al., 1991; Graner, 2004). The MWG2287 locus is closely linked to the barley *gai* mutation in the centromeric region of barley chromosome 2H (Börner et al., 1999). We speculate that the *gai* (*sdw3*) locus, described by Börner et al. (1999) and Gottwald et al. (2004), may correspond with a major plant height QTL associated with the cluster of DNA markers near the CNL045 locus of LG2a. The CNL045 loci are associated with high-density clusters of markers on *Leymus* LG2a and LG2b linkage groups, probably caused by reduced recombination near the centromere. Yang et al. (1995) also described a partially dominant *GA-ins* dwarfing gene on the short arm of chromosome 2A of wheat (*Rht21*), which could be homeologous to the *gai* mutation mapped by Börner et al. (1999). In any case, the barley, wheat, and *Leymus* plant height genes on homeologous group two are evidently different from the so-called "green revolution" *GA-ins* dwarfing genes of wheat, barley, and rice encode gibberellin response modulators that map to Triticeae group four (Peng et al., 1999).

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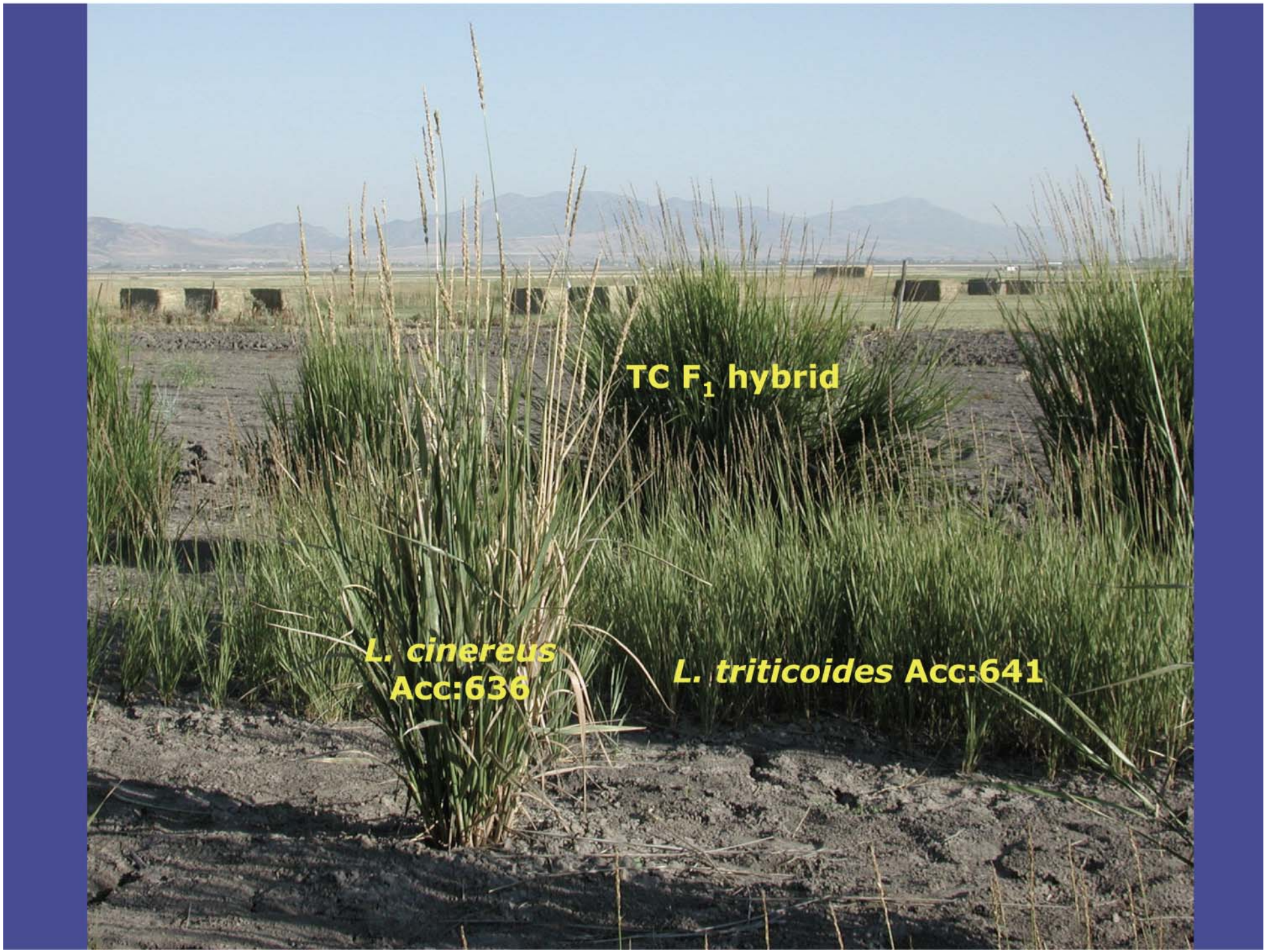
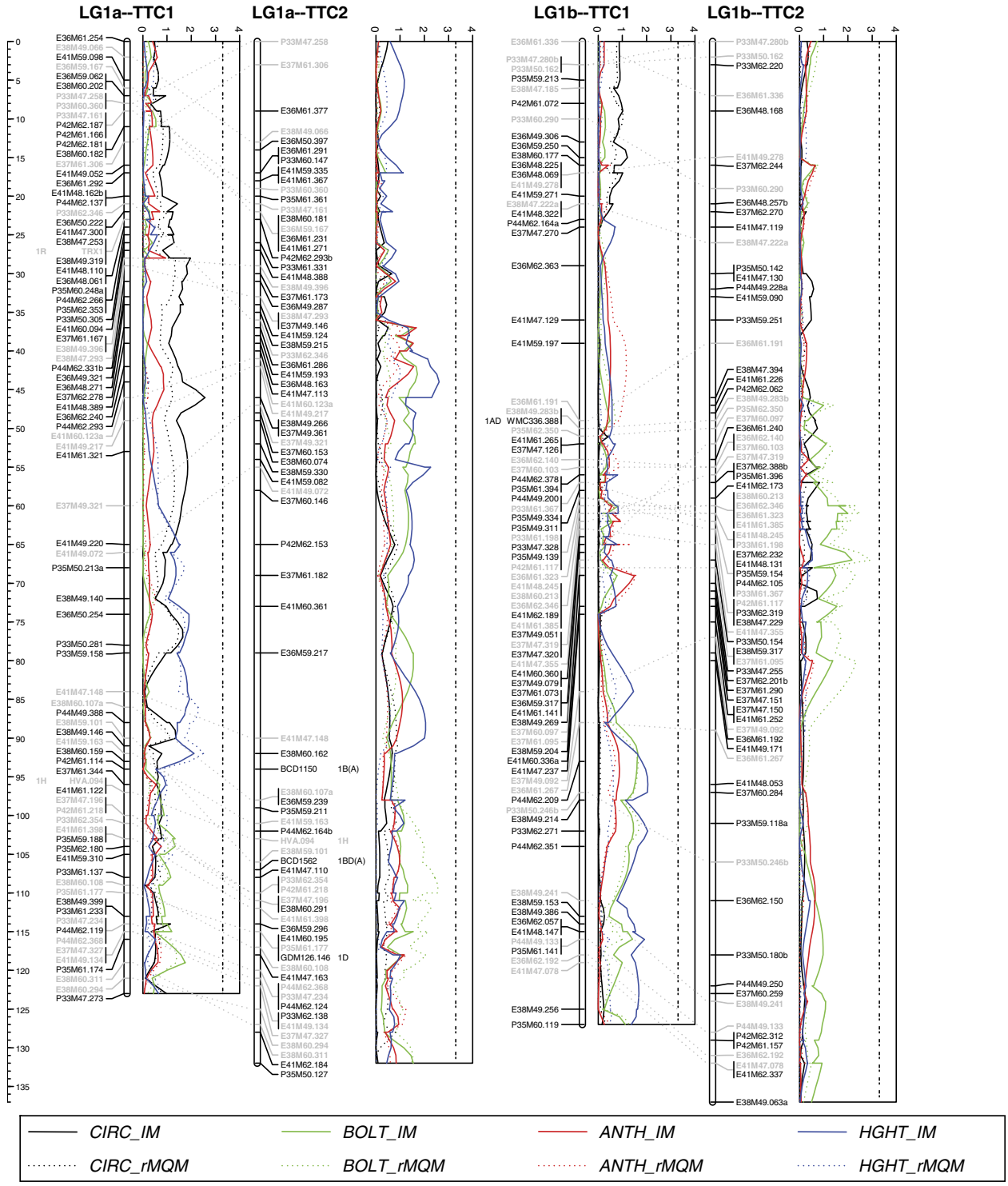
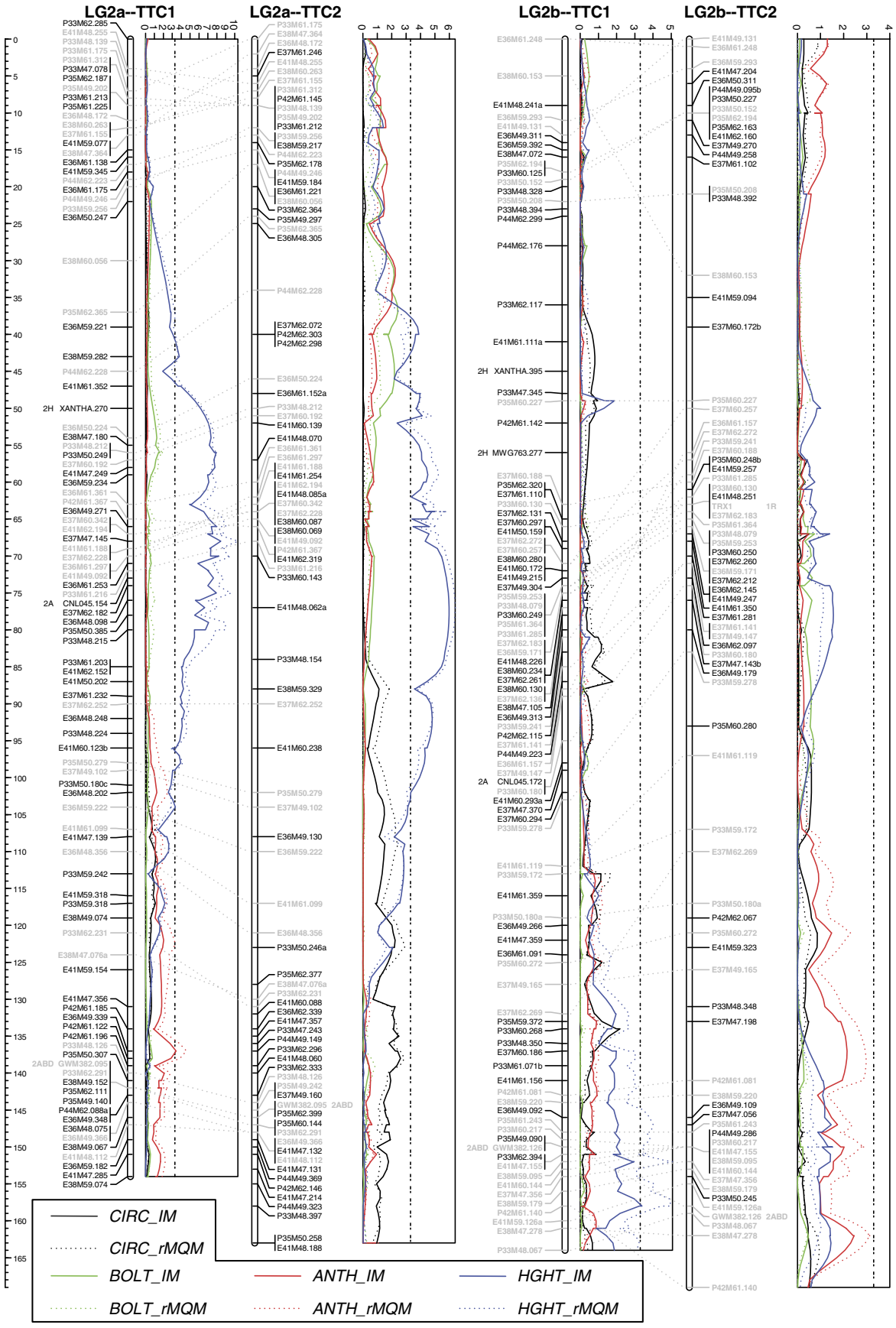
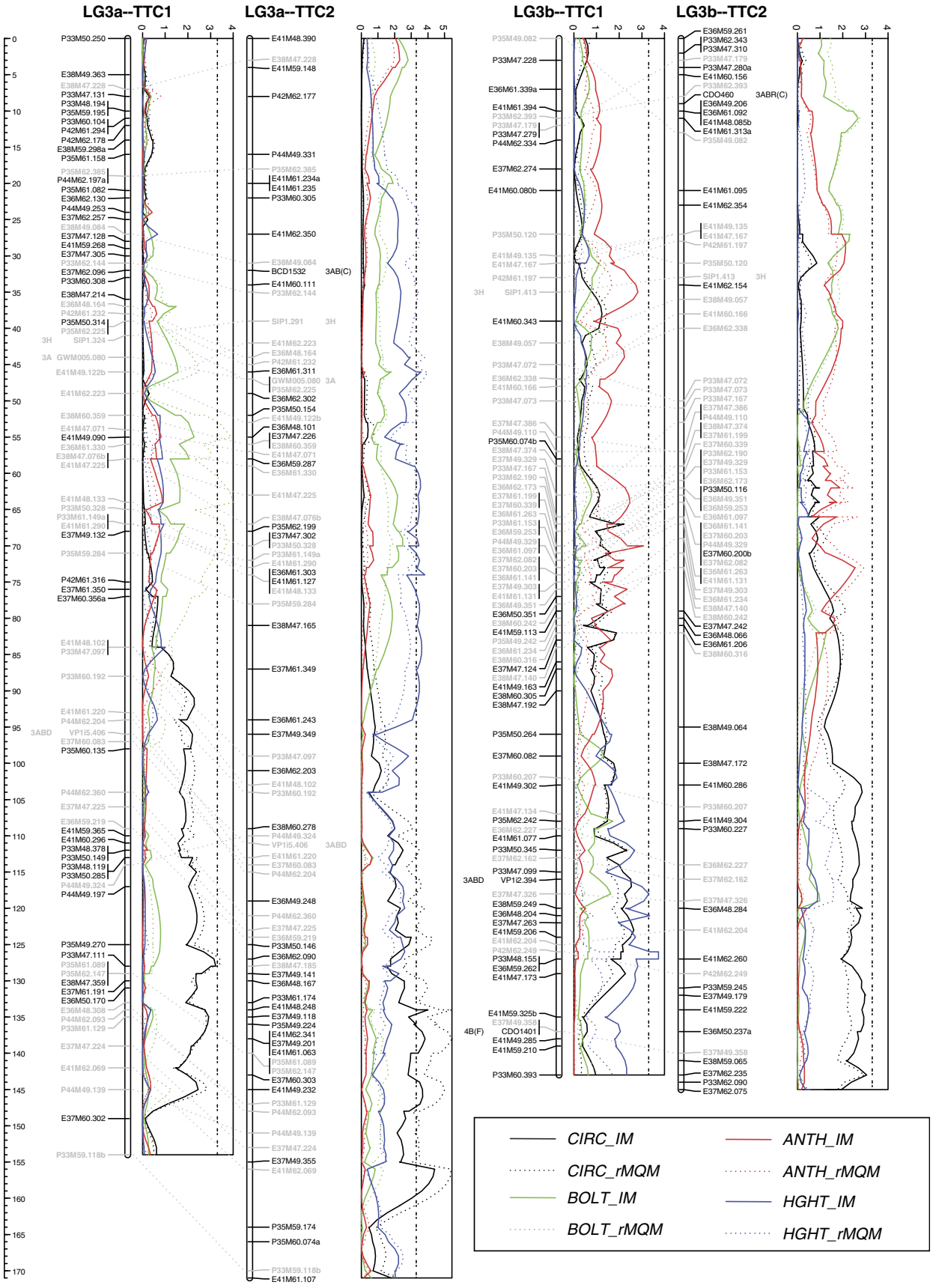
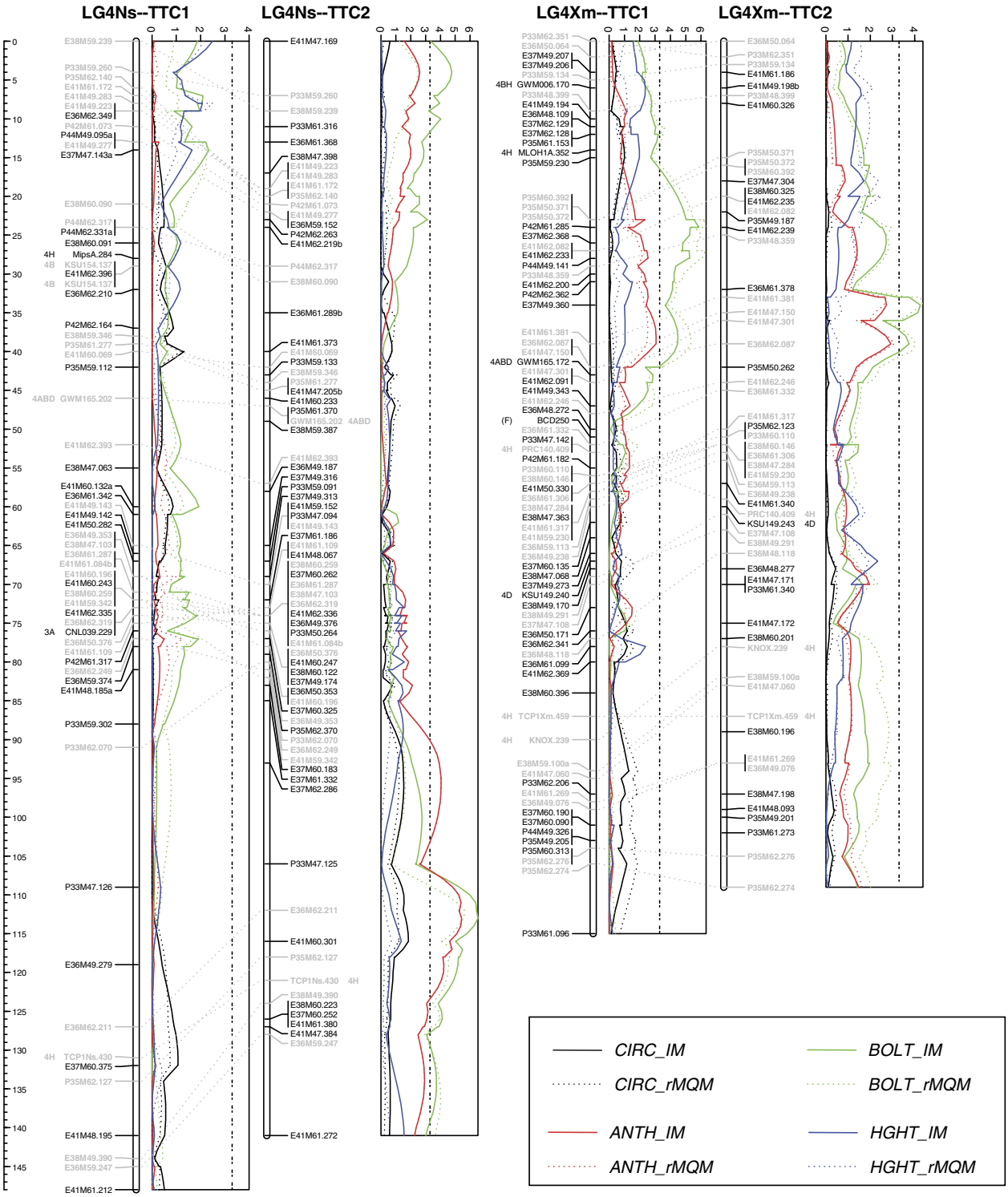


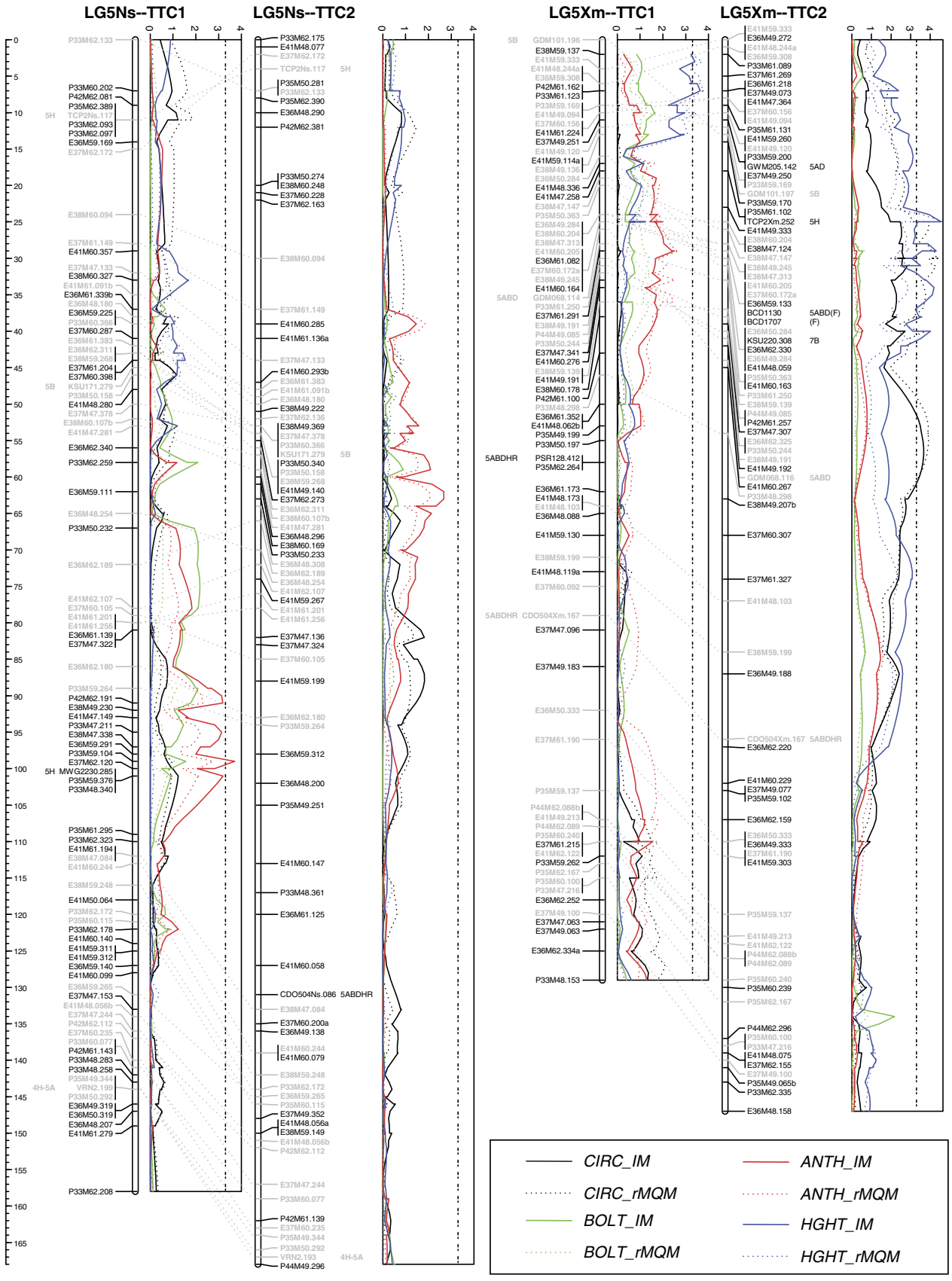
Genome-wide QTL interval mapping (IM) and restricted multiple QTL model (rMQM) mapping in the full-sib *Leymus* TTC1 and TTC2 families based on log of the odds (LOD) for circumference of plant spreading (CIRC), proportion of bolting culms (BOLT), anthesis date (ANTH), and plant height (HGHT) based averages over 2002, 2003, and 2004. A threshold value of 3.3 LOD is shown for reference.

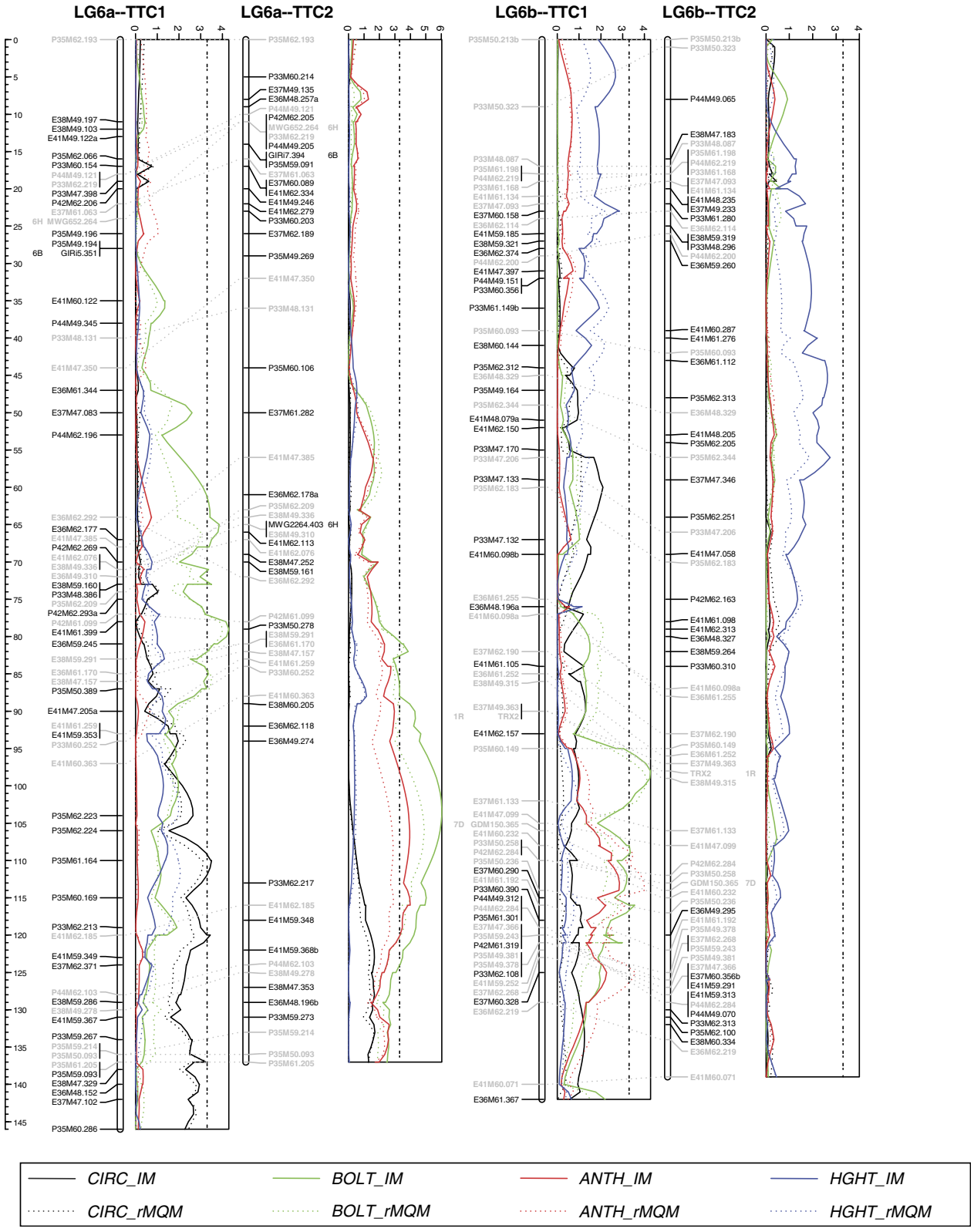


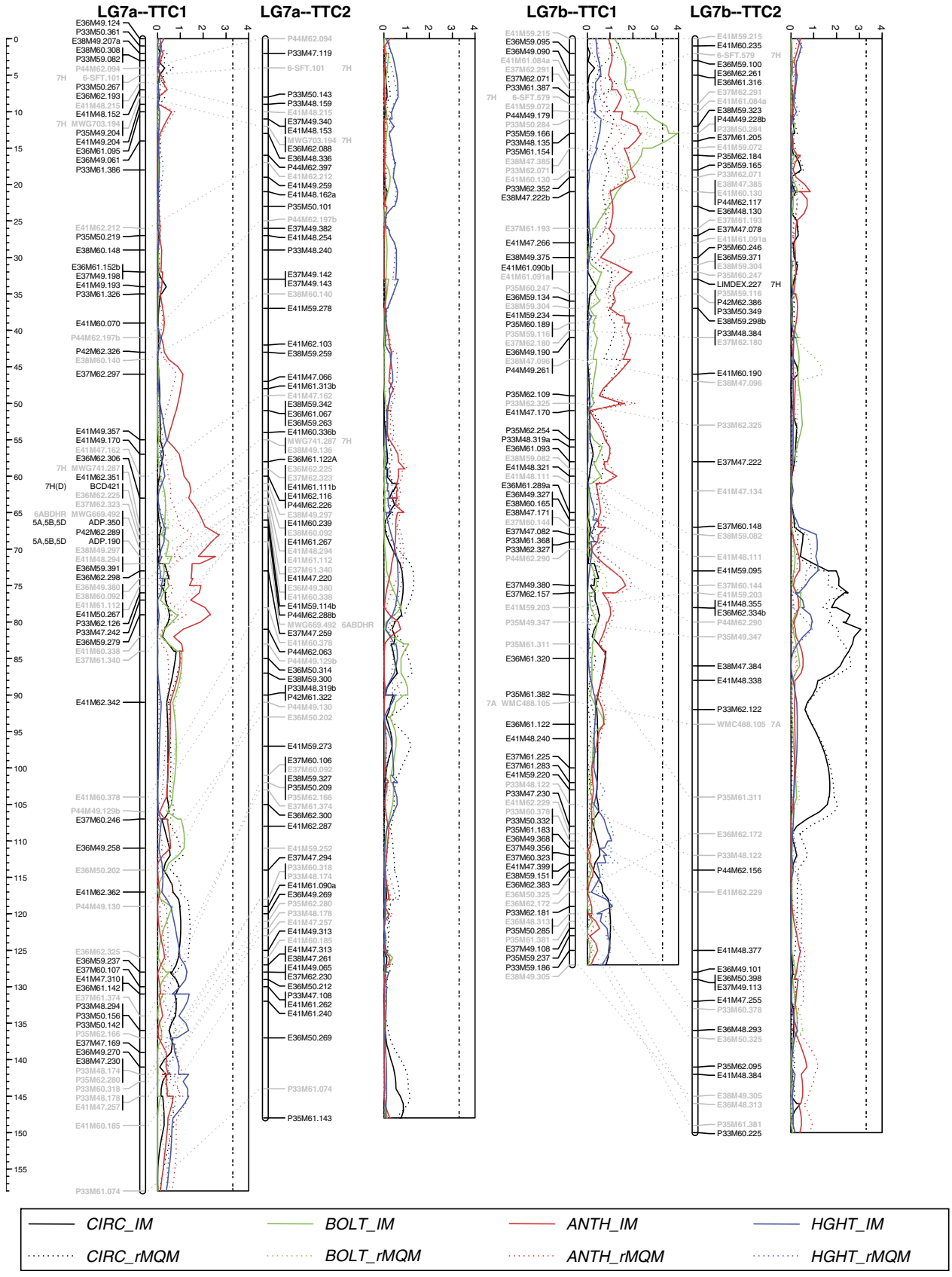




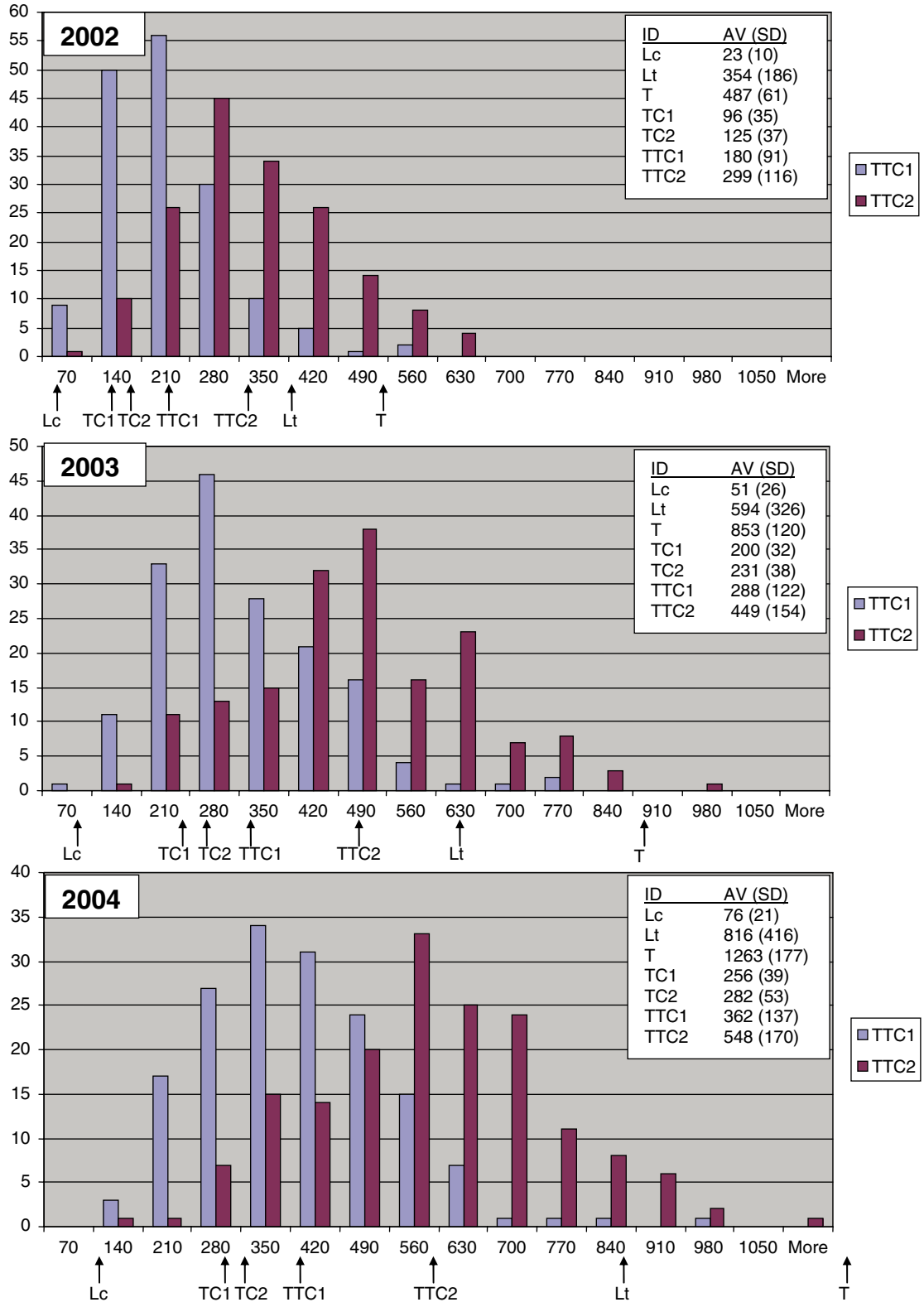




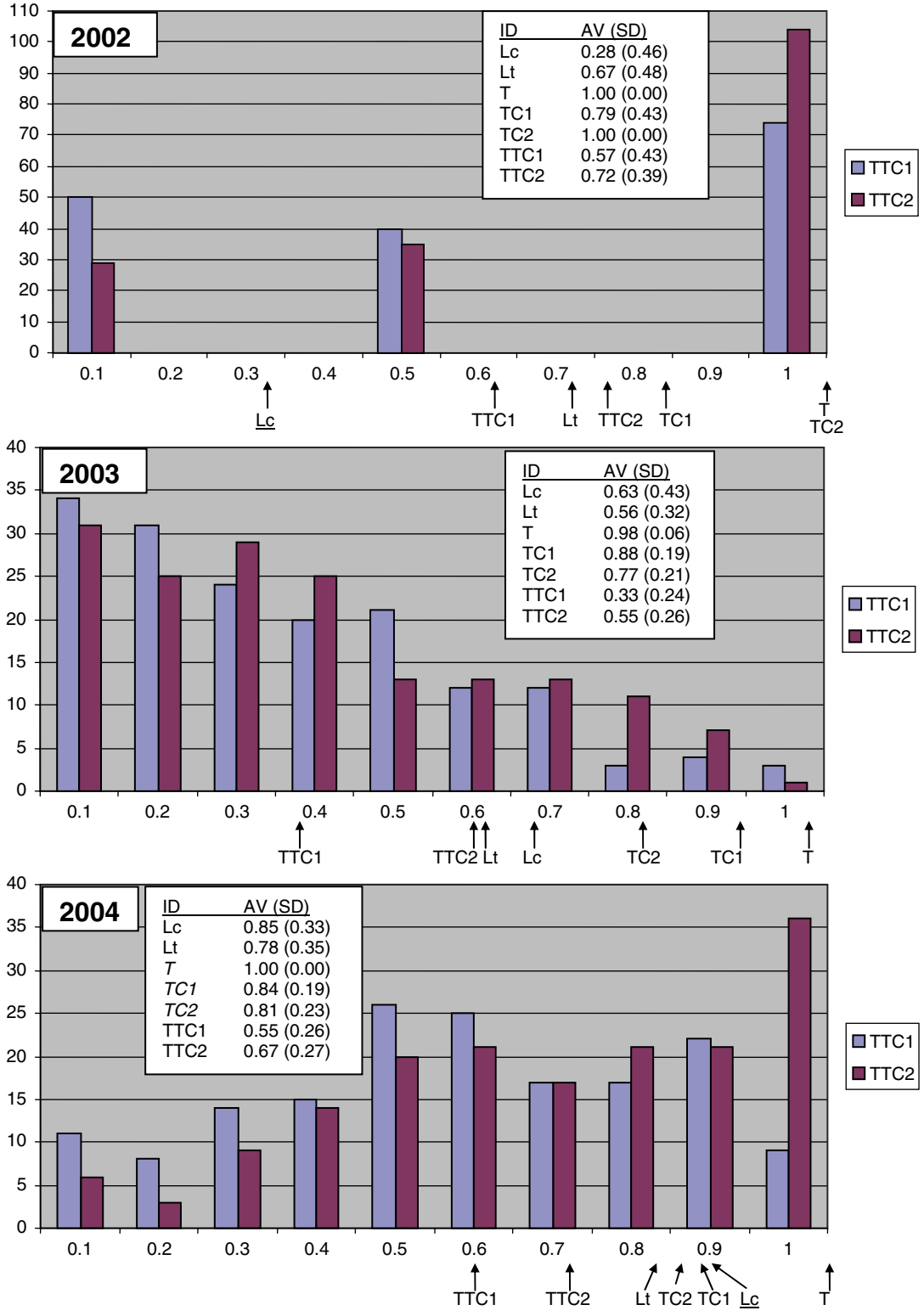




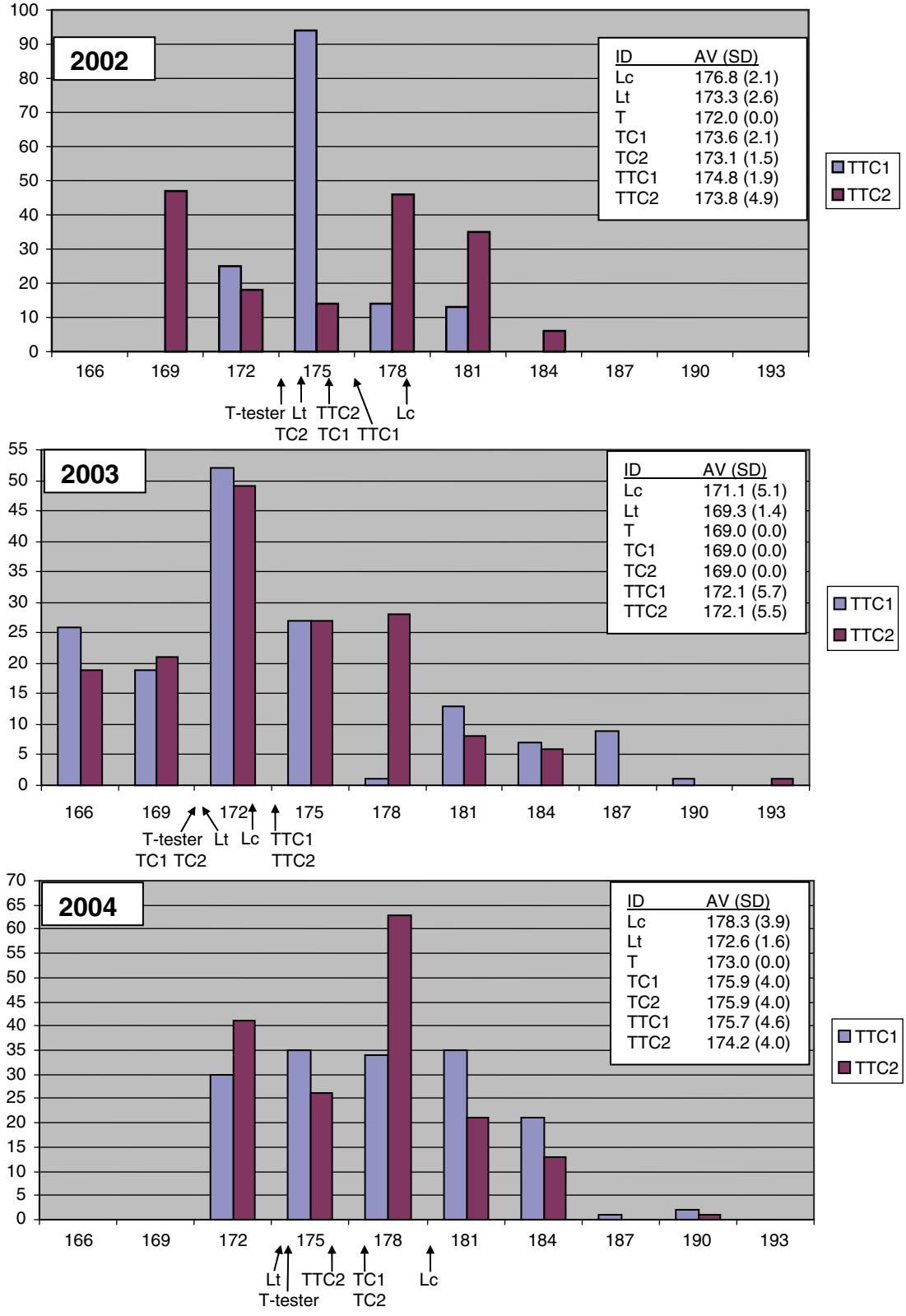
Histograms for circumference (cm) of plant spreading in the full-sib *Leymus* TTC1 (n=164) and TTC2 (n=170) mapping families, based on means of two clones, compared to the heterogeneous *L. cinereus* Acc:636 (Lc) and *L. triticoides* Acc:641 (Lt) progenitors, interspecific hybrid parents (TC1 and TC2), and recurrent parent (*T*).



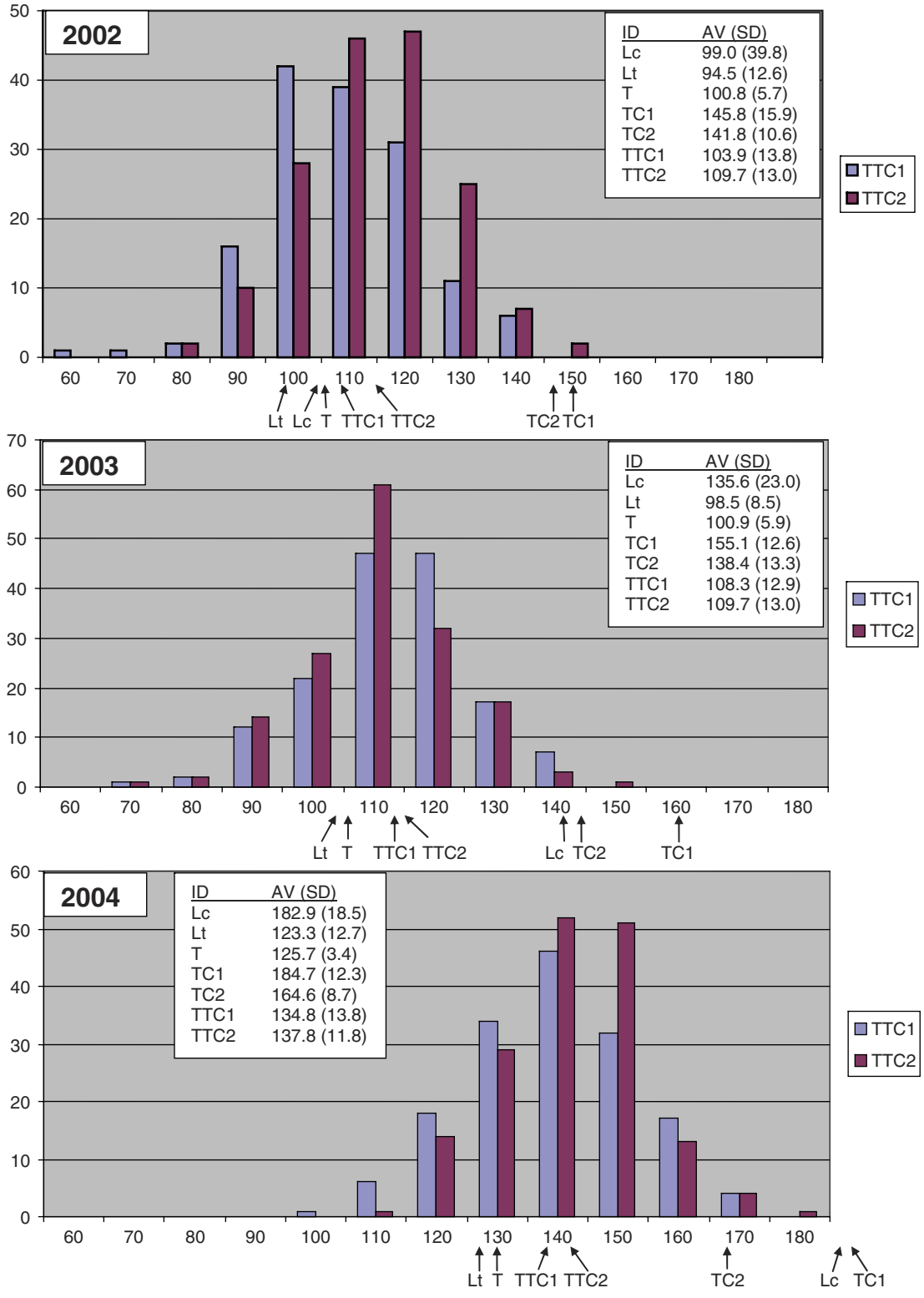
Histograms for proportion of bolting culms in the full-sib *Leymus* TTC1 (n=164) and TTC2 (n=170) mapping populations, based on means of two clones, compared to the heterogeneous *L. cinereus* Acc:636 (Lc) and *L. triticoides* Acc:641 (Lt) progenitors, interspecific hybrid parents (TC1 and TC2), and recurrent parent (T).



Histograms for anthesis date (days from January 1) in the full-sib *Leymus* TTC1 (n=164) and TTC2 (n=170) mapping populations, based on means of two clones, compared to the heterogeneous *L. cinereus* Acc:636 (Lc) and *L. triticoides* Acc:641 (Lt) progenitors, interspecific hybrid parents (TC1 and TC2), and recurrent parent (T).



Histograms for plant height (cm) in the full-sib *Leymus* TTC1 (n=164) and TTC2 (n=170) mapping populations, based on means of two clones, compared to the heterogeneous *Leymus cinereus* Acc:636 (Lc) and *L. triticoides* Acc:641 (Lt) progenitors, interspecific hybrid parents (TC1 and TC2), and recurrent parent (T).



Description of additional SSR (CNL and KSU) and STS markers added to the *Leymus* TTC1 and TTC2 maps originally published by WU *et al.* (2003)

Loci(groups)	Primers	Gene or motif	Anchor loci	Expected amplicon	Mapped amplicons§
TRX1 (LG1a, LG2b)	GGATAACATGACCCTAAAAACT† GTTGTGCATCACAGGGTTATA	AY204511 thioredoxin, Xbm2,	1R	287‡ Leymus	289
TRX2 (LG6B)	GGATAACATGACCCTAAAAAG† TTGTTCATCACAGGGTTATC	AY204511 thioredoxin, Xbm2	1R	287‡ *Leymus*	288
CNL45 (LG2A, LG2B)	GAGAGAGCTTCGTCCCACTC CACCACTCGTTTCTTTCACTTT	$(ga)_9$, putative RNA binding protein	2A	167‡ wheat	154, 161, 170, 172
KSU154 (LG4Ns)	GGAGACTCTGGTCATCTCGC ATACTGGAGTGAAGGCACGG	$(cac)_7$	4B	146‡ wheat	137
CNL39 (LG4Ns)	TACCTGTGCGGCGATGAAT CAGGAGCAGGAGAACGTGAA	AF251264 $(atgc)_5$ rubisco activase B	3A	220‡ wheat	229
BCD1117 (LG4Ns, LG4Xm)	TCAGTTCTCAATAGAAGTGCTGTG TCCTGAATAAGGTCTTCATACCAA	BCD117 barley cDNA clone	4HD	209	152, 211
HVCABG (LG4Ns)	ACACCTTCCCAGGACAATCC CAGAGCACCGAAAAAGTCTGTA	Rubisco $(AT)_{29}$	4H	182 barley	152
HVM068 (LG4Xm)	AGGACCGGATGTTCATAACG CAAATCTTCCAGCGAGGCT	$(GA)_{22}$	4H	204 barley	199
KSU149 (LG4Xm)	GAGCCACCAGAGCAGAAATC CGAGCTCCCCTTCTTCTTCT	$(acc)_9$	4D	228‡ wheat	240, 243
KSU171 (LG5Ns)	TCTTGCTTGCATTGTAACCG TCATGTCTGGGAGCATGGTA	$(agt)_7$	5B	243‡ wheat	279
MWG2230 (LG5Ns)	AATGATGTTGCTTTCCTGTTTGCTC ACAGATGATGATGGCGTGCAGCTTT	MWG2230 barley genomic DNA	5H	320 barley	285
VRN2 (LG5Ns)	AGTACCAGTTCTTCRCCCAAGG CTGCASYAGGTGAGCCAT	Wheat vrn2 (AY485644)	4H, 4A/5A	203 wheat	193,199
MWG2264 (LG6a)	AGGTAGAAGTCAAACTGTGTGGGAT GTATTACTTTACGAGTTAGATGCTA	MWG2264 barley genomic DNA	6H	400-450	403
ADP (LG7a)	CCTCCGTGAACAATTTCCTG TCCAATACGAGCATTCTTGT	M31616 ADP glucose phosphorylase	5ABD	1003‡ rice	350 *Taq*I 190 *Taq*I

† FAM 5' labeled primer.
‡ Amplicon size based on sequence data.
§ Amplicon size based on comparisons with internal size standards in capillary electrophoresis.